The influence of cognitive biases on psychophysiological vulnerability to stress

Katherine Elizabeth Randall

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University of East Anglia, Norwich

Norwich Medical School

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Declaration of Contribution of Work

Studies one, five, and six were designed by Katherine Randall (with guidance from supervisors). Studies two and three were collaboratively designed by Katherine Randall and Dr Bristow (Anglia Ruskin University). Study four was collaboratively designed by Katherine Randall, Dr Bristow (Anglia Ruskin University), and Drs Dunn and Brodbeck (Cognition and Brain Sciences Unit, Cambridge).

Data collection for studies one, two, five, and six was carried out exclusively by Katherine Randall. Data was collected for study three by Lauren Barrett (undergraduate), with guidance from Katherine Randall and supervision by Dr Bristow. For study four, data collection was started (17 participants of which 5 were excluded from analysis) by Charlie Powell (undergraduate) and completed by Katherine Randall (74 participants of which 5 were excluded from analysis).

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"If you're not failing every now and again, it's a sign you're not doing anything very innovative." - *Woody Allen*

Abstract

Individuals who disproportionately attend to negative aspects of a situation (attention bias), or who unduly interpret ambiguity in a negative manner (interpretive bias) report more psychological ill-effects of stress than those with balanced or positively-skewed inclinations. Cognitive Bias Modification (CBM) techniques improve maladaptive biases through implicitly-based association learning, with induced positive biases buffering the future perception of stress. Six experimental studies investigated the next stage of this link to bolster and significantly enhance the clinical potential of CBM; how natural and modified biases influence the biological response to acute stress. Studies 1-3 established reliable protocols associated with using laboratory stress tasks and measuring salivary stress biomarkers. Studies 4-5 investigated links between natural and trained biases on psychological and biological stress responses. Study 6 tested the immediate robustness of CBM training. While psychological and physiological stress responses were initiated, attentional biases were not found to moderate acute biological stress responses. Conversely, interpretive biases were related to the recovery from the acute stress and positive interpretive training led to a faster biological recovery from acute stress in high test-anxious individuals relative to sham training. However, neither bias was found to moderate the psychological response to stress. Further, evidence emerged to caution a more selective use of CBM. Positive interpretive training led to a more negative bias and slower physiological recovery to stress in individuals with low trait anxiety or inherent positive biases. From these results, information processing biases are proposed to have less influence on genuinely stressful events but, instead, govern the extent to which unthreatening situations are perceived as stressful. Consequently, negative biases are hypothesised to cause unnecessary and excessive perceptions of stress, resulting in chronic hyper-activity. Combined CBM-A/I tools are recommended to jointly realign maladaptive biases, enabling an effective, efficient, but transitory physiological response to real stress.

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List of Abbreviations

Bias-related

ABI	Attentional bias index
CBM	Cognitive bias modification
CBM-A	Attentional bias modification
CBM-I	Interpretive bias modification
IBI	Interpretive bias index

Psychological Disorders

GAD	Generalised Anxiety Disorder
MDD	Major Depressive Disorder
PTSD	Post Traumatic Stress Disorder
SAD	Social Anxiety Disorder

General

CAT	Cognitive ability tests (refers to a stressor task in Chapter 7)
CBSU	Cognition and Brain Sciences Unit
H-TA	High test anxious (refers to a participant group in Chapter 7)
L-TA	Low test anxious (refers to a participant group in Chapter 7)
NA	Negative affect
NHS	National Health Service
OCam	Ostracism Camera (refers to a stressor task in Chapter 6)
PA	Positive affect
SM	Social manipulation (refers to a stressor paradigm in Chapters 3 and 6)
SOS	Salimetrics oral swab
TAL	Tissue Analysis Laboratory

TES	Threat Evaluation System
TMB	Tetramethylbenzidine

Questionnaires

A-RSQ	(Adult) Rejection Sensitivity Questionnaire
DASS	Depression Anxiety Stress Scale
FNE	Fear of Negative Evaluation
GHQ	General Health Questionnaire
IPIP	International Personality Item Pool
ISEL	Interpersonal Support Evaluation List
ISS	Interpersonal Sensitivity Scale
PANAS	Positive and Negative Affect Scale
PSS	Perceived Stress Scale
SACL	Stress Arousal Check List
SADS	Social Avoidance and Distress Scale
STAI	State Trait Anxiety Inventory
TAI	Test Anxiety Inventory
TAS	Test Anxiety Scale
TMAS	Taylor Manifest Anxiety Scale
VAS	Visual Analogue Scales

Stress/physiology-related

ACTH	Adrenocorticotrophic hormone
ANS	Autonomic nervous system
CAR	Cortisol awakening response
CRF	Corticotrophin-releasing factor
GAS	General adaptation syndrome

HPA	Hypothalamic-pituitary-adrenal
IgA	Immunoglobulin A
sAA	Salivary alpha amylase
SAM	Sympathetic-adrenal-medullary

Stressor tasks

MIST	Montreal Imaging Stress Test
TMCT	Trier Mental Challenge Test
TSST	Trier Social Stress Test

Units of measurement

µl	Microlitres
M	Molar
ml/min	Millilitres per minute (flow rate)
ms	Milliseconds
nm	Nanometres
RPM	Revolutions per minute
U/min	Units per minute (alpha amylase secretion)
U/ml	Units per millilitre (alpha amylase activity)
µg/dl	Micrograms per decilitre (cortisol concentration)

1.0 CHAPTER ONE

INTRODUCTION

1.1 Overview

Over the last 25 years, much attention has been dedicated to the relationship between cognitive biases and anxiety. This journey started by researchers noting a positive correlation between cognitive bias and anxiety, with tendencies to focus predominantly on negative aspects of a situation or interpreting ambiguity principally in a negative manner being associated with higher levels of anxiety (e.g. MacLeod, Mathews, & Tata, 1986, Butler & Mathews, 1983, respectively). In an effort to explore the issue of causation, researchers developed computerised programmes that successfully modified natural attentional and interpretive biases (MacLeod, Rutherford, Campbell, Ebsworthy, & Holker, 2002, Grey & Mathews, 2000, respectively). These researchers found that training individuals towards a more positive or negative bias led to changes in anxiety vulnerability to subsequent stressful events (e.g. Wilson, MacLeod, Mathews, & Rutherford, 2006). Since then, the field has been flooded with studies replicating these effects in different contexts (e.g. study venue, method of delivery, see Beard 2011 for a review), and has recently demonstrated its potential in clinical settings (e.g. Schmidt, Richey, Buckner, & Timpano, 2009).

Before these cognitive bias modification (CBM) methods can be introduced as standalone clinical tools, there remains certain largely blank areas of investigation. One such area concerns the extent to which the anxiety-bias relationship exists on a biological level, that is, whether threat biases affect our physiological stress systems in the same manner as our psychological stress systems. It seems logical to assume that cognitive biases do on some level predict how individuals respond on both a psychological *and* physiological scale, though there is currently little data to conclude this either way. As over-active biological

systems have been linked to adverse mental and physical health, finding a method of reducing this activity (i.e. through bias modification) could further the clinical potential of CBM. The overall objective of the research in this thesis is to explore the link between attentional and interpretive biases and psychophysiological vulnerability to acute stress. This objective shall be addressed by monitoring responses from the two main stress pathways (hypothalamic-pituitary-adrenal and sympathetic axes) and investigating the influence of natural and trained cognitive biases on psychological and physiological stress responses to acute stress paradigms.

This introductory chapter will discuss the concept of physiological stress, including perception and response. Literature on attentional and interpretive cognitive biases will then be presented, which will cover knowledge of links between cognitive biases and anxiety, recent efforts aimed at establishing a link between cognitive biases and psychophysiological stress, and the potential for CBM to modify emotional and physiological vulnerability to stress. Finally, specific aims and hypotheses of the thesis shall be presented.

1.2 Stress

1.2.1 Conceptualising Stress

The concept of ‘stress’ is nowadays a well represented and familiar topic in the media, in health and lifestyle recommendations, and in routine everyday conversations. Due to its constant use, the term has become somewhat ambiguous in meaning. In modern science the term ‘stress’ is commonly used to refer to external forces (e.g. an environmental factor such as an exam), internal states (e.g. feeling tense), or physical responses (i.e. how the body reacts).

Selye (1936) was amongst the first to operationalise the concept of stress in a psychological sense and defined it as "the nonspecific response of the body to any demand made upon it" (p. 32). Selye argued that every individual exhibited a non-specific three stage physiological response to every challenge, which he termed the general adaptation syndrome (GAS; Selye, 1976). Following the perception of stress, individuals enter the first stage, alarm, during which an organism initiates a physiological response. This stage is similar to Cannon's (1929) fight or flight theory, with physiological activation serving to prepare the body with energy to either contest the stressor (fight) or flee the threat (flight). A key aspect of this alarm phase is that the response to demand is generic across organisms and situations, positive or negative, a point which has received considerable criticism over the years (e.g. McEwen, 2005). Where stressors persist, organisms enter the second stage of Selye's GAS model; coping and resistance. During this stage Selye postulated that internal systems adapt to the stressor to reduce its impact. While the initial effects of the stressor reduce or disappear during this stage, the organism is more susceptible to other stressors. As these coping capacities are finite, where an individual's ability to cope is exceeded by the persistence or amplification of the stressor the third stage, exhaustion, occurs. During this stage, the initial effects of the stressor reappear due to a depleted capacity to counter them, leading to illness and possibly death.

Of principal importance in Selye's (1976) GAS model was the concept of maintaining a homeostatic balance. While stage one - the physiological response to acute stress – is still thought to be valid, the secondary stages have been subjected to reinterpretation over the years. For example, McEwen (2005; McEwen & Wingfield, 2003) distinguished between the terms homeostasis, a balance of physiological variables (e.g. temperature) that are essential for life, and allostasis, the process of resuming homeostatic balance. McEwen claimed that stage 1 of Selye's model represented an initial allostatic effort which, if sustained, resulted in

an allostatic state (stage 2 of GAS). Allostatic states consisted of physiological and behavioural changes aimed at restoring homeostasis. Failure to fulfil this aim ultimately resulted in allostatic load or overload (stage 3 of GAS). This final reinterpreted stage presents the largest disparity to Selye's model as, while stage 3 of the GAS was always considered harmful, McEwen noted that this stage resulted in the collective effects of allostatic states that could either be adaptive or maladaptive. For example, allostatic loads (adaptive) might be illustrated by an animal that has gained considerable body weight prior to hibernation. Alternatively allostatic overloads (maladaptive) might arise following random environmental extremes (e.g. natural disasters) which leaves an organism susceptible to disease.

1.2.2 Physiological Response to Stress

While Selye documented various physiological changes in each stage of the GAS model, many have since been outdated and so have not been noted here. At present, it is a generally agreed upon notion that individuals exhibit a physiological reaction to an event perceived as stressful and that, as Selye postulated, this is a non-specific reaction. This acute physiological response consists of a dual activation of two key stress systems; the sympathetic-adrenal-medullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axes (e.g. Charmandari, Tsigos, & Chrousos, 2005; Yang & Glaser, 2002). The SAM axis forms part of the autonomic nervous system (ANS), which is controlled by the hypothalamus and is responsible for regulating a range of physiological activity, such as heart rate, digestion, and blood pressure. The ANS is comprised of the sympathetic and parasympathetic branches, which generally work together in an antagonistic manner with parasympathetic dominance during times of rest. SAM activation provides a relatively immediate effect, commonly referred to as the 'fight or flight' response (Cannon, 1929), during which there is a more dominant sympathetic tone. Contemporary understanding of Cannon's work argues that sympathetic arousal serves to redirect energy to systems that might be most useful to combat

the challenge, such as increased blood flow to muscles rather than digestive tracts, and increased heart rate (Galosy, Clarke, Vasko, & Crawford, 1981). On encountering a high level of stress, HPA activation occurs involving a sequence of hormonal changes. Corticotrophin-releasing factor (CRF) is secreted from the paraventricular nucleus of the hypothalamus stimulating the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland. ACTH travels through the blood to the adrenal glands, directing the release of glucocorticoids, such as cortisol. A major function of cortisol is to act on reserves of glucose (glycogen) to release the stored energy (Clow, 2001). The HPA complex operates on a negative feedback loop, during which the released glucocorticoids act back on the first stages of the hormonal transmission to suppress the further release of CRF and ACTH.

This thesis is concerned with capturing acute psychological and physiological responses to stress. The literature so far introduced has related to the physiological stress response. Prior to considering the subjective role of stress perception, such as cognitive buffers and individual differences that place an individual at a greater or lessened risk of eliciting a physiological stress response, it is important to first discuss how the physiological response will be represented.

1.2.3 Capturing the Physiological Stress Response

Cortisol. As an end product of HPA activation, cortisol has become the hormone that is most frequently studied in the assessment of the physiological stress response. Following secretion, cortisol circulates throughout the body in the bloodstream. After a 15 minute delay, free cortisol (that which remains physiologically active rather than being bound to proteins) enters the saliva through the cellular membranes (Aardal-Eriksson, Karlberg, & Holm, 1998). For researchers, salivary cortisol provides a practical, less costly, and minimally-invasive mode of measurement relative to serum cortisol, and shares a stronger correlation with serum

ACTH (Aardal-Eriksson et al., 1998). For this reason, salivary cortisol arguably provides a better indicator of HPA activation compared with serum cortisol.

Levels of cortisol in the circulatory system (excluding exogenous activation) are regulated by diurnal rhythms (Kirschbaum & Hellhammer, 1989). Typically, cortisol levels significantly rise after awakening (Horrocks et al., 1990) to a peak approximately 30-45 minutes after waking (Pruessner et al., 1997). This profile, commonly known as the cortisol awakening response (CAR), is present from very young infancy (under one years of age; de Weerth, Zijl, & Buitelaar, 2003) and is thought to remain stable over time (Pruessner et al., 1997), though can be affected by stress. For example, Kunz-Ebrecht, Kirschbaum, Marmot, and Steptoe (2004) found evidence of a larger CAR (i.e. greater release of cortisol) in participants on workdays compared to weekend days. This was not found to be linked to time of awakening, which has previously been considered as an influential factor (e.g. Kudielka & Kirschbaum, 2003). Instead, Kunz-Ebrecht et al. proposed the differences to be due to occupational demands experienced on workdays, during which participants reported significantly greater levels of stress and significantly poorer mood. This conclusion linking stress to the CAR is shared among many eminent researchers within the field (e.g. Pruessner, Hellhammer, Pruessner, & Lupien, 2003; Schlotz, Hellhammer, Schulz, & Stone, 2004; Wust, Federenko, Hellhammer, & Kirschbaum, 2000). Following the initial rise, cortisol levels gradually decline for the remainder of the day (Edwards, Evans, Hucklebridge, & Clow, 2001).

Studies focusing on changes in cortisol to infer HPA activation in biobehavioural research typically place saliva collection points prior to a procedure (e.g. an acute stress task) and at several time points following the task. The first collection point acts as a baseline measure against which subsequent samples are compared to monitor change over time. Increases in cortisol have been documented following a range of laboratory stressors, such as

forced exposure to unpleasant graphic stimuli (e.g. Nejtek, 2002; Takai et al., 2004), extreme temperatures (e.g. al'Absi, Petersen, & Wittmers, 2002; Andreano & Cahill, 2006), social rejection (e.g. Blackhart, Eckel, & Tice, 2007; Stroud, Salovey, & Epel, 2002), and the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993), which is a task that is specifically tailored to combine various stressful elements (e.g. Fiocco, Joober, & Lupien, 2007; Schommer, Hellhammer, & Kirschbaum, 2003).

Factors that affect cortisol levels include (although are not limited to) caffeine (e.g. Lovallo et al., 2005), nicotine (e.g. Stalke et al., 1992), alcohol (e.g. Badrick et al., 2008), and strenuous physical exercise (e.g. Usui et al., 2011). Certain traits, such as personality or traits linked with personality (e.g. aggression), have also been shown to influence cortisol release (e.g. Oswald et al., 2006; Pruessner et al., 1997). For this reason, where possible, such factors should be controlled or measured in laboratory studies.

Alpha Amylase. Only relatively recently, salivary alpha amylase (sAA) has started to receive attention as a possible indicator of sympathetic activation. Though it's primary function is to aid the digestive process (Baum, 1993), this enzyme has been found to mirror stress-induced changes in noradrenaline following sympathetic activation (Chatterton, Vogelson, Lu, Ellman, & Hudgens, 1996; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). However, while there is a general consensus that sAA increases following a range of acute stressor tasks (e.g. Allwood, Handwerger, Kivlighan, Granger, & Stroud, 2011; Bosch et al., 1996; Nater et al., 2005, 2006; Rohleder et al., 2004; van Stegeren, Wolf, & Kindt, 2008; Wetherell et al., 2006), more recent research suggests that the relationship between sAA and noradrenaline is not as analogous as first envisaged. For example, Nater et al. (2006) identified increases in both variables following the induction of stress (using the TSST), though additionally noted that correlations between the two parameters were not statistically significant. Similar findings have been demonstrated by Wetherell et al..

Alternatively, studies have demonstrated significant correlations between sAA reactivity and other measures of sympathetic activation, such as skin conductance (El-Sheikh, Erath, Buckhalt, Granger, & Miza, 2008) and aspects of cardiovascular reactivity (Nater et al., 2006). In light of this and the robust findings of stress-induced increases in sAA, current opinion within the field considers sAA to reflect sympathetic activation more generally rather than noradrenaline specifically.

Prior to literature indicating the potential of sAA, there was no established reliable method of monitoring sympathetic activation from a saliva sample as the transfer of noradrenaline itself into saliva takes approximately 60 minutes, which makes it near impossible to accurately map any stress-induced variation (Kennedy, Dillon, Mills, & Ziegler, 2001). While it was possible to assess noradrenaline activity through serum samples, the invasive nature of the collection procedure acted as a potential confound to researchers. Since its introduction, sAA has quickly emerged as a popular choice for researchers investigating the area of stress primarily as it enables assessment of the two major physiological stress response systems (the SAM and the HPA axes) reliably through one parameter (e.g. Engert et al., 2011; Granger et al., 2007).

Produced in the acinar cells of the parotid saliva gland, one of the three major glands responsible for the production and secretion of saliva (Humphrey & Williamson, 2001), a potential confound in the measurement of sAA concerns whether its concentration in saliva is influenced by changes in flow rate. Being flow rate dependent implies that changes in the rate at which saliva is secreted leads to direct changes in the levels of enzymes and hormones found within saliva. This matter is important in consideration of the fact that, while noradrenaline increases follow sympathetic activation, flow rate is governed predominantly by parasympathetic command (Anderson et al., 1984; Garrett, 1987). Therefore, without clarification of this relationship, it would not be possible to determine whether changes in

sAA concentration reflect a sympathetic or parasympathetic response thereby limiting the enzymes biomarker potential. While initial investigation into the matter suggests that the two are independent (e.g. Rohleder, Wolf, Maldonado, & Kirschbaum, 2006), more recent exploration suggests otherwise. For example, Beltzer et al. (2010) identified a significant inverse relationship between sAA activity and flow rate. This demonstrates that the two variables might be linked. Owing to the matter not being fully resolved, current specialist advice recommends controlling for saliva flow as a potential confound when measuring sAA (Salimetrics LLC, 2012).

The natural activity of sAA is subject to circadian variation in a manner that appears in direct opposition with cortisol rhythms (Ghiciuc et al., 2011). Nater, Rohleder, Schlotz, Ehlert, and Kirschbaum (2007) first profiled the diurnal patterns of the enzyme in 76 participants (group composed of a mixed gender), and documented a steep fall in activity within the first 30 minutes of waking, followed by a general increase in activity over the day. As with cortisol, Nater et al. established significant links between reported chronic stress and the awakening response of sAA; higher levels of chronic stress were associated with greater levels of sAA. This pattern has also been documented in groups of participants who experience the chronic stress of PTSD. Upon awakening, Thoma, Joksimovic, Kirschbaum, Wolf, and Rohleder (2012) noted increases in sAA in PTSD sufferers rather than the typical decrease exhibited by healthy controls.

Several additional exogenous factors are known to exert an acute influence on sAA and, thus, need to be controlled in research. These include nicotine (e.g. Zappacosta et al., 2002), caffeine (e.g. Bishop, Walker, Scanlon, Richards, & Rogers, 2006), alcohol (e.g. Enberg, Alho, Loimaranta, & Lenander-Lumikari, 2001), exercise (e.g. Chatterton et al., 1996), and, as would be expected due to its supportive role in digestion, food intake (e.g. Messenger, Clifford, & Morgan, 2003).

1.2.4 The Perception of Stress

Studies that measure the physiological response to acute stress paradigms often do so to discern subjective factors that influence an individual's perception of (and, thus, their response to) stress. While Selye (1976) remains broadly correct in his notion of a non-specific response, he gave no consideration to individual differences that make an individual more or less vulnerable to the ill-effects of stress. As such, he assumed that every organism responds to every environmental challenge in the same manner. This conjecture holds the organism as a passive, almost robotic, entity in the process of being stressed.

More recent transactional models of stress focus almost exclusively on these subjective factors that serve to mediate vulnerability to stress. For example, Lazarus and Folkman (1984) highlight appraisal and coping strategies as key factors in determining what situations evoke a stress response, and the extent of that response. On detecting threat, individuals are suggested to undergo a primary appraisal of potential challenges in which personal risk is calculated, prior to a secondary appraisal in which individuals evaluate their capacity to manage the challenge. Similarly, Cox and Mackay's (1981) transactional model claims that stress occurs as a result of perceived demands exceeding an individual's perceived capacity to manage them. Since stress models have emerged that emphasise the role of the individual in determining subjective sensitivity to stress, much effort has been invested into identifying cognitive mediators of stress. For example, the perception of control over certain aspects of a potentially stressful situation has been shown to have a buffering effect on the development of depressive symptomology in adolescents (Deardorff, Gonzales, & Sandler, 2003). Another factor that has received steadily increasing amounts of interest over the past decade as a possible mediator to stress surrounds the notion that certain internal cognitive biases dictate the extent to which individuals preferentially divide their information processing resources in the face of ambiguity.

1.3 Cognitive Biases

The term *cognitive bias* was first used by Zajonc (1960; Zajonc & Burnstein, 1965) to refer to automatic assumptions made based on incomplete information. This early meaning of the term appears to have held, though it was not until much later that efforts were made to investigate the effects of these biases on emotions. In 1979 Teasdale and Fogarty found that, following positive or negative mood induction, participants recalled memories that were analogous to their current mood (i.e. positive or negative in content) significantly quicker than those that conflicted with their current mood (e.g. a positive memory following negative mood induction). Teasdale and Fogarty proposed that these findings were due to a recall bias, in which current mood rendered memories of a corresponding nature to be more accessible while incongruous memories were less accessible. Mathews and Bradley (1983) additionally found that mood induction tended to influence reporting of depressive episodes, with negative mood induction being linked to a higher volume and more severe symptomology recall. These studies led to the comparison of individuals who differed in their levels of trait anxiety to investigate whether similar patterns of response were found.

1.3.1 Attention Biases and Anxiety

In 1986, MacLeod, Mathews, and Tata developed a task designed to objectively measure the extent to which individuals attend to positive and negative materials. The visual probe task involves two words being simultaneously presented for a short time on screen above and below a central fixation point. One word denotes a negative meaning while the other is neutral or positive in meaning. Typically, after 500 milliseconds the words disappear and a probe (e.g. a left or right facing arrow) appears in the spatial location of one of the words. Participants are required to respond to the probe (e.g. identify the direction the arrow points). This procedure continues for a number of trials. Individuals who are generally faster

to respond to probes that are positioned in the prior location of negative words relative to positive words are argued to have a negative bias, as their attention was automatically drawn to the more negative of the stimulus enabling them a identify the probe quicker. MacLeod et al. found a clear distinction for individuals who had been referred for training in anxiety management to preferentially focus their attention towards negative words. Alternatively matched control participants with more typical levels of anxiety displayed a preference towards neutral words.

Variants of the visual probe task have been frequently employed in studies investigating attention biases, with the links between negative attentional bias and anxiety being replicated in many different samples and settings. Bradley, Hogg, White, Groom, and de Bono (1999) demonstrated this effect in a clinically anxious sample. Using the visual probe method, individuals suffering from generalised anxiety disorder (GAD) were found to disproportionately attend to pictures of faces that displayed negative emotions over neutral faces. The link between anxiety and attention has also been demonstrated in more specific phobias. For example, Lavy, van den Hout, and Arntz (1993) found a significantly higher tendency for spider-phobic participants to attend to words relating to spiders relative to generally negative words or neutral words. This link between anxiety and attention appears very robust in the published literature, with only a handful of studies having been included here. To give an idea of the breadth of this finding, Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, and van IJzendoorn (2007) reviewed 172 studies that looked at this link and concluded that the association between anxiety and attention was indeed reliable.

In a study that significantly progressed the authenticity of the cognitive bias' proposed influence, MacLeod and Hagan (1992) demonstrated a potential for attentional bias to act as a predictor for subsequent emotional distress following a stressful event. Higher anxiety was matched with a negative attentional bias for female participants awaiting a cervical screening.

For participants who subsequently received a cervical pathology diagnosis, preconscious attention to threat was also found to significantly predict the intensity of their emotional response. This effect has since been replicated by van den Hout, Tenney, Huygens, Merkelbach, and Kindt (1995), who showed the effect in a group of participants not currently undergoing a high degree of stress.

1.3.2 Interpretive Biases and Anxiety

Following on from the early research investigating recall bias and mood, Butler and Mathews (1983) focused on the encoding phase rather than the recall phase of information processing. They found that anxious individuals tended to interpret ambiguous materials in a more threatening manner than less anxious individuals. Further, high anxiety was linked with a propensity for focusing more on threatening than non-threatening material. In parallel to work investigating attentional biases, researchers also set out to investigate the link between biased interpretive cognitions and anxiety. Using a similarly simple yet effective technique as that used to investigate attentional bias, Mathews, Richards, and Eysenck (1989) established similar anxiety-bias effects dependent on how individuals interpreted emotional ambiguity. To achieve this, Mathews et al. superset a series of homophones – words with both positive and negative connotations (e.g. bury/berry) – into a list of words matched in terms of familiarity and length characteristics. Participants were presented the word list in an auditory fashion and were required to write down the word they heard. While all participants showed a preference for the threatening interpretations, there was a clear difference in interpretive bias between high and low anxious groups. Participants who had been referred for anxiety management training (i.e. the high anxiety group) reported the threatening interpretation of homophones significantly more frequently than matched control participants.

An alternative manner of measuring biases of interpretation involves presenting participants with an emotionally ambiguous sentence and requiring them to either solve the final word of the sentence (e.g. Hirsch & Mathews, 1997) or read a subsequent sentence that related to each meaning (positive and negative) respectively (e.g. MacLeod & Cohen 1993). For example, a statement might read "The doctor examined little Emily's growth" (MacLeod & Cohen, 1993). Participants would then be presented with the sentences "Her height had changed since her last visit" (positive) or "Her tumour had changed since her last visit" (negative). Importantly, the two sentences differ only in terms of the disambiguation word (*height* or *tumour*). A faster reading speed in reading negatively valenced endings would therefore be attributed to a negative interpretive bias, as the meaning would be more congruent with the reader's understanding of the scenario.

Using the methods described above (or similar) researchers have, again, repeatedly shown evidence to support the existence of anxiety-dependent interpretive biases. For example, Hazlett-Stevens and Borkovec (2004) demonstrated a tendency for participants with GAD to automatically associate ambiguous homographs (words that have multiple meanings though are spelt the same, e.g. batter) with their negative meaning relative to non-anxious participants. Alternatively, Stopa and Clark (2000) showed that participants with social phobia interpreted ambiguous scenarios that depicted social situations significantly more negatively that control participants. As with attentional biases, therefore, it seems the effects are well-documented and appear relatively robust.

1.3.3 Model of Cognitive Bias

Developments in the field of cognitive biases have largely been based on empirical evidence rather than being derived through theoretical models. Of the models that do exist (e.g. Öhman, 1993; Wells & Mathews, 1994; Williams, Watts, MacLeod, & Mathews, 1997),

one stands out for its attempts to explain biased information processing both in attention and interpretation using one model. Mathews and Mackintosh (1998) posited that individuals possess a Threat Evaluation System (TES), which serves to reinforce/abate certain features of a situation that compete for processing resources. Critically, the features need to differ in terms of whether they do or do not represent any threat. Consider, for example, an emotional Stroop task (e-Stroop; Gotlib & McCann, 1984), in which participants are required to determine the colour of a printed word. As a modification to the original Stroop task (Stroop, 1935), the printed words contained within an e-Stroop task are emotionally valenced. According to Mathews and Mackintosh's model, when faced with such a trial, several aspects of the stimuli are processed in parallel (e.g. colour of word, word identification, meaning of word). In such an instance these features are devoted attention based on various factors, such as personal significance, conscious effort, or primed inclinations. The TES further prioritises these competing attributes to determine which receives the limited attentional resources. Those that match the encoded system are given a higher priority relative to incompatible cues. Further, Mathews and Mackintosh theorised that activation from the TES was positively correlated with anxiety. Musa, Lépine, Clark, Mansell, and Ehlers (2003) found support for this by demonstrating how individuals high in anxiety showed a poorer performance in naming the colour of the threat-related word on the e-Stroop relative to low anxious individuals. This can be explained by a larger interference from the TES assigning attention to threat-related features (i.e. meaning of the word) at the expense of the non-threat features (i.e. colour).

By incorporating an element of conscious and effortful control into their model that is capable, to a point, of overriding interference from the TES, Mathews and Mackintosh's model accounts for why not every potential threat dominates processing resources. From an evolutionary perspective, the TES provides a necessary manner of attending and responding

to danger. All individuals will allocate information processing resources to threat cues when they represent a severe enough danger. Biases in information processing start to develop as a result of repeated interactions between competing attributes and a tendency for the TES to dominate processing resources. For example, individuals who are more responsive to threat cues will, over time, develop a wider portfolio of threat representations and conditioned responses. Consequently, these processing biases can leave individuals vulnerable towards further anxiety as future threat cues are consistently given precedence even when they might only represent a relatively mild threat.

1.3.4 Cognitive Bias and the Physiological Stress Response

Although the relationship between cognitive biases and emotional stress (e.g. anxiety) has been well documented and appears robust, considerably less research has investigated the link between bias and physiological stress. It would seem reasonable to assume that cognitive biases might influence the extent to which individuals physically respond to stress by altering their perception of and, thus, response to it. As negative biases have been matched with higher levels of anxiety relative to positive biases (e.g. MacLeod, Mathews, & Tata, 1986; Mathews, Richards, & Eysenck, 1989), it is plausible to expect that negative biases might also be more closely linked to states of physiological hyper-arousal, in which stress systems are overworked, that are associated with high/clinical levels of anxiety (e.g. Mantella et al., 2008) relative to positive biases.

To explore the link between cognitive biases and the physiological stress response, Fox, Cahill, and Zougkou (2010) adopted a visual probe task to test attentional biases. As a slight alteration to the convention use of this task, male participants were required to respond to a probe appearing in the spatial vicinity of a previously displayed picture (rather than emotive word). Pictures were selected for their arousing content (either positive, negative, or

neutral) and were presented for either 14 milliseconds or 300 milliseconds before being masked by a random reconstruction of the picture which was displayed until a total time of 500 milliseconds had passed. Four months after the initial test of attention bias, participants were exposed to an acute laboratory public speaking stressor in which they were instructed to give a short (5 minute) speech on the necessity of statistics in psychology. A further four months later, participants were required to repeat this process, this time with the topic relating to their perceived preparations for their impending exams. Fox et al. found that a preconscious attention bias (i.e. in trials where the picture was displayed for just 14 milliseconds) to negative stimulus was predictive of cortisol reactivity on both acute stressor tasks. Considering the 8 month delay between initial bias measurement and subsequent stressor exposure, this finding appears to demonstrate a clear and stable link between bias and physiological reactivity.

These results are similar to those of van Honk et al. (2000) who showed that preconscious attentional biases towards negative pictoral stimuli was associated with significant cortisol increases to the task. However, van Honk et al. also noted a similar significant association when the pictoral stimuli were presented within conscious threshold. Further, though not directly measuring interpretive biases per se, Gaab, Rohleder, Nater, and Ehlert (2005) have demonstrated the predictive power of cognitive appraisal processes for predicting cortisol responses to the TSST. Gaab et al.'s study suggests that the manners in which an individual perceives a situation (i.e. as threatening/non-threatening) directly influences their response to it.

Dandeneau, Baldwin, Baccus, Sakellaropoulo, and Pruessner (2007) have demonstrated a link between interpretive bias and physiological vulnerability to stress. Interpretive bias was measured using a modified visual probe task that used pictures of faces that either portrayed a positive or negative expression. Stress was induced using the Montreal

Imaging Stress Task (MIST; Dedovic et al., 2005), a combined stressor derived from the Trier Mental Challenge Test (TMCT; Kirschbaum, 1991) in which participants have to solve mental arithmetic problems (academic element) within a set time whilst receiving criticising feedback from the researcher (social element). Results showed a significantly positive relationship between bias and responses to the MIST; participants who produced a greater cortisol response also demonstrated a significant attentional bias towards negative faces.

1.4 Cognitive Bias Modification

The findings discussed above demonstrate a clear cognitive bias for individuals who are more susceptible to anxiety to both attend to and interpret ambiguity in an overly threatening manner. However these studies predominately used correlation designs. As one of the first studies to address the issue of causation, MacLeod, Rutherford, Campbell, Ebsworthy, and Holker (2002) used a modified version of their dot-probe task which served to train rather than test attentional bias. Rather than positioning the probe behind both the positive and negative words equally, it was consistently placed behind either the neutral or negative word. This alteration was designed to encourage participants to develop an implicit rule in which they learnt to automatically attend to stimuli of a certain valence (i.e. neutral or negative) when both were presented. Indeed, participants were subsequently found to be faster at responding to target probes when the location of the probe matched their training condition. Further, MacLeod et al. found that this training affected individual vulnerability to subsequent stress. Participants who were assigned to the attend-negative condition were found to respond to a greater extent to a combined laboratory stressor relative to those in the attend-neutral condition. The stressor consisted of an anagram task (academic challenge), which participants completed whilst being videotaped. Participants were informed that the videos might then be used for later class demonstrations to illustrate particularly good or bad performance (socio-evaluative element). The significance of this finding is further

underscored by that fact that the two groups showed no difference in their response to the same stressor prior to the attention training procedure.

The finding that it was possible to experimentally manipulate an individual's cognitive bias enabled researchers to examine the relationship between cognitive bias and anxiety by investigating the issue of causation. Understandably, clarification of this issue held a great deal of appeal to supporters of the field, who proposed the potential clinical importance of their work. In 2000, Grey and Mathews further extended the field by developing a laboratory technique that successfully trained participants towards a more negative interpretive bias. This was achieved by forcing the participant to repeatedly generate negative meanings of a series of homographs. Homographs are words that have two meanings despite the same spelling. Grey and Mathews selected a series of homographs for which one meaning was unpleasant while the other was neutral. For example, the word "batter" could refer to an uncooked mixture (neutral) or to the process of hurting someone (negative).

Motivated by the research in the area of attentional bias, Wilson, MacLeod, Mathews, and Rutherford (2006) sought to replicate similar emotional vulnerability patterns using a training programme aimed at interpretive biases. Participants were trained to automatically associate ambiguity in a positive/neutral or negative manner (depending on their condition) using homograph training (Grey & Mathews, 2000). Participants then underwent a stressor in which they watched video clips that portrayed footage of emergency rescues. As Grey and Mathews had found, the interpretive training was found to be effective in modifying individuals' bias. Further, and corresponding to findings relating to attentional biases, this training appeared to successfully moderate emotional responses to the stressor. Participants who had received positive training reported significantly smaller increases in anxiety following the stressor relative to negatively trained participants.

Coinciding with the development of homograph training, another method emerged that followed on from the ambiguous scenarios interpretive test (Hirsch & Mathews, 1997). Mathews and Mackintosh (2000) developed an ambiguous scenarios training task, which worked by presenting participants with a series of descriptions of situations. The situations presented a relatively ambiguous setting to encourage participants' natural biases to start operating. However, the final sentence of the scenario was presented in a manner that resolved the situation either in a positive or negative manner. Mathews and Mackintosh demonstrated the success of this technique by successfully training participants toward a more positive or negative bias.

Using the ambiguous scenarios training, Mackintosh, Mathews, Yiend, Ridgeway, and Cook (2006) continued down the route of demonstrating the positive effects of modifying cognitive biases. Participants received training directed at improving or worsening their biased interpretations of ambiguity. The following day, participants completed a task that was designed to measure their biased interpretations. This included participants reading 10 descriptions of scenarios that contained an element of ambiguity. For example, a situation could involve sitting waiting for your doctor to read out some test results and noticing the doctor chatting to a colleague holding your file. After the 10 scenarios have been presented, participants are required to recall them in turn and rate four sentences according to their recollection of how the scenario was presented. One sentence referred to a real positive interpretation (e.g. the doctor is saying the tests are normal), one a positive foil (e.g. the doctor is pointing out your impressive fitness rating), one a negative real interpretation (e.g. the results describe bad news), and one a negative foil (e.g. the doctor is making fun of your chart). Participants were found to show biased interpretations in line with their previous training condition. Further, subsequent exposure to a stressor (watching a graphic accident video) again revealed the buffering effects of positive interpretive training.

1.4.1 The Potential of Bias Modification

With further developments in the area, the effects of cognitive bias modification (CBM) training have been found to endure over a 24 hour period (Yiend, Mackintosh, & Mathews, 2005), endure changes in testing environments (Mackintosh, Mathews, Yiend, Ridgeway, & Cook, 2006) and generalise to new domains (from social to academic anxiety; Salemink, van den Hout, & Kindt, 2010). In response to these advances, researchers have been quick to start investigating the applied potential of CBM. For example, the finding that training can effectively alter cognitive bias has been reproduced repeatedly (e.g. Salemink, van den Hout, & Kindt, 2009; Steinman & Teachman, 2010). Moreover, bias modification methods appear effective in significantly reducing anxiety in clinical populations, including populations suffering from generalised anxiety disorder (GAD; Amir, Beard, Burns, & Bomyea, 2009), generalised social phobia (Amir, Beard, Taylor et al., 2009), major depressive disorder (MDD; Joorman, Hertel, LeMoult, & Gotlib, 2009), and social anxiety disorder (SAD; Schmidt, Richey, Buckner, & Timpano, 2009).

Following the volume of articles demonstrating its potential, attempts have also been made to explore the utility of these training programmes outside of the laboratory in a home environment. Blackwell and Holmes (2010) adopted a home-based training paradigm in which participants who were currently experiencing a major depressive episode were instructed to imagine themselves in a series of scenarios that were presented in an auditory fashion. The scenarios remained ambiguous until the end of the paragraph, after which they consistently resolved into a positive outcome. Participants listened to 64 scenarios on a daily basis for five consecutive days. Results showed improvements in over half of the sample (seven participants), with improvements persisting over a two week period. See, MacLeod, and Bridle (2009) have also revealed encouraging findings with their home-based attention modification programme using a real-life stressor. Singaporean participants completed visual

probe training everyday for 15 days prior to relocating to Australia to continue tertiary education. Participants who received positively valenced training reported significantly less anxiety arising from the stressful life event relative to participants in the no training group. These studies provide a particularly persuasive argument for the potential of CBM as, prior to this study, findings had largely been laboratory-based. By demonstrating external validity with an easy-to-access programme, Blackwell and Holmes and See et al. significantly advanced the field in its drive towards clinical application.

1.4.2 CBM and the Physiological Stress Response

More recently, interest has progressed onto investigations into the relationship between cognitive biases and the physiological stress response. In consideration of the fact that many psychopathological disorders develop from a hypersensitive tendency towards stress, research into methods designed to augment the manners in which participants respond to stress seems an area worthy of attention. Dandeneau, Baldwin, Baccus, Sakellaropoulo, and Pruessner (2007) argued that attentional processes are significantly involved in perception of and response to stress. Using a group of telemarketers, an occupation in which workers regularly encounter the stressful experience of rejection, Dandeneau et al. (experiment 3b) tested this proposition. Participants were required to complete attention modification training for five consecutive days. Training consisted of a series of trials in which participants had to locate a head shot photo of a person expressing a positive emotion (e.g. smiling) in a 4x4 matrix of head shot photos of people expressing negative emotions (e.g. frowning or scowling). Scowling faces were designed to represent rejection which, as previously mentioned, featured heavily within participants' job roles. Results indicated that participants who completed the find-the-smile training released significantly less cortisol over a working day and had significantly lower peak cortisol reactivity relative to participants in the comparison condition, who had completed a control find-the-five-petaled-flower (in a 4x4

matrix of seven-petaled flowers) task. This study provided the first account of a potential link between CBM and psychophysiological vulnerability to stress. However, although the study used a natural stressor, there was no baseline measurement of physiological activity against which to compare the observed training effects.

To date, there are no existing studies that have focused on the influence of biased cognitions on sAA. However, research conducted by Schartau, Dalgleish, and Dunn (2009) suggests there is a potential for such a link to exist. Schartau et al. utilised a slightly different form of re-training cognitive biases that focused on reappraising the negative interpretations based on four general themes. Participants were required to practice this method of re-appraisal whilst watching distressing films (training group) or watch the films without practicing any form of emotion regulation (comparison group). Participants in the training group showed a reduced electrodermal response (a marker of sympathetic activation) in response to an ensuing distressing film relative to participants in the comparison condition. This study demonstrates that it is possible to modify the sympathetic physiological impact of stress by changing how individuals interpret the situation. Therefore, as a measure of sympathetic activation, sAA should also be sensitive to such modifications.

Summary

The evidence presented above outlines a robust relationship between cognitive biases (natural or trained) and emotional vulnerability to stress. Negative biases are linked with greater anxiety both in normal and clinical samples, while positive biases are matched with lower levels of anxiety. Further, training is effective in modifying the ways in which individuals attend to and interpret threat. Generally, individuals who are trained towards a more positive way of processing information appear more resilient on a psychological scale to subsequent episodes of acute stress. Alternatively, training directed towards a poorer bias

leads to greater negative impact of ensuing stressful episodes. So far, these findings present an organised account of influences underlying subjective psychological susceptibility to stress, though few studies have sought to reproduce the effects on a biological scale. As such, little is known as to whether reducing the propensity to psychologically perceive threat through CBM will incur any improvements to health associated with over-active physiological stress systems. Studies that have started to investigate this have documented positive findings, though considerable further research is necessary to understand the influences and implications of information processing biases on a physiological scale.

1.5 Focus and Rationale for this Thesis

Increasingly greater numbers of the population appear to be negatively influenced by stress to a point where it disrupts their daily lives. For example, a national Labour Force Survey found that 35% of all work-related sickness was attributed to "stress" in 2010/11, with an estimated 5.4 million days work lost (Health and Safety Executive, 2011). As discussed in this chapter, the perception of stress has been found to negatively impact natural physiological rhythms. For example, chronic stress has been associated with a high release but blunted diurnal profile of cortisol (e.g. Miller, Chen, & Zhou, 2007; Tseng, Iosif, & Seritan, 2011). While acute cortisol release serves to provide a temporary solution to challenges by, for example, liberating stored reserves of energy (Clow, 2001), chronically elevated levels have been linked to an increased risk of cardiovascular disease (Whitworth, Williamson, Mangos, & Kelly, 2005), type-II diabetes (Dallman, 2010), and poorer immune defences (McEwen, 2000). Further, various forms of psychopathology are known to be linked to an overactive biological stress system (e.g. Plotsky, Owens, & Nemeroff, 1998; Pruessner, Hellhammer, Pruessner, & Lupien, 2003; Vreeburg et al., 2009). In light of this, there is a clear rationale for attempts to be made to try and identify simple and effective methods of reducing the impact of daily stress.

Cognitive biases appear to share an undeniable link to how people respond to their surrounding world. People who seem to predominantly decipher their environment in a negative manner seem more at risk of suffering the psychological ill-effects of stress, such as increased anxiety. Alternatively, less sensitivity to threat appears to act as a buffer to the psychological manifestations of stress. The research discussed above has demonstrated promise for CBM methods to change habitual information processing biases. However, to date the literature linking information processing biases to perceived stress has relied too heavily on self-report measures of changes in emotion, which expose findings to criticism of reporting biases. One way of validating this research is to identify similar effects on a biological basis. Research that has started to investigate this has identified a tentative link between the ways in which individuals physiologically respond to stress and their biased cognitions. However, to date there are only a handful of studies dedicated to this cause. Further, this link appears disproportionately supported by research that focuses on biases in attention. The aim of this thesis is to explore this association further focusing *both* on biases in attention and interpretation. Studies will endeavour to isolate robust links between attentional and interpretive biases and an individual's sensitivity to acute stress. Further, efforts will be made to investigate the effects of CBM on the physiological stress response. Ultimately, this thesis aims to validate the existence of an authentic link between information processing biases and the psychophysiological stress response using objective (physiological) and subjective (psychological) measures of stress, and to further CBMs potential as a clinical tool.

The next chapter will outline principles and methods involved in assessing biological stress markers in saliva. Following on from this, chapters will be dedicated to six experimental studies that aim to establish reliable designs (Studies 1-3), which can be put to

use to explore the relationship between naturally occurring and modified cognitive biases and the psychophysiological stress response (Studies 4-6).

2.0 CHAPTER TWO

Saliva Storage and Analysis

2.1 Tissue Analysis Laboratory

The Tissue Analysis Laboratory (TAL) is based at Anglia Ruskin University, Cambridge, and was the site of all saliva analysis presented in this thesis. The TAL has standardised techniques for assaying cortisol levels but, at the time of initial collaboration, had no established method of assaying sAA. In parallel to the studies presented in this thesis, work was conducted to develop and test an in-house sAA assay protocol for use in the TAL (see Appendix I for further details involved in this process).

2.2 Sample Preparation

Samples are always frozen at -80°C immediately after the study session until analysis. On the day of analysis, samples are removed from the freezer and defrosted in a biosafety class II cabinet. Once fully defrosted, samples are spun in a centrifuge at 1500RCF for 15 minutes. If using Salimetrics Oral Swabs (SOS), the insert and swab are then removed and placed in 2% Virkon for disinfection prior to disposal.

2.3 Cortisol Assay

Principle. This competitive immunoassay uses a microtitre plate that had been pre-coated with monoclonal antibodies to cortisol. This produces binding sites that are sought by cortisol in the sample (or standards or controls, which are regulated to act as assay controls) and known amounts of cortisol linked to conjugate (horseradish peroxidise) in competition. Following an incubation phase, excess conjugate and unbound sample cortisol are washed away before a substrate is added to the wells, resulting in the well developing a blue colour. After a specified amount of time, the reaction is stopped by the addition of acid to the well.

The resulting yellowish colour intensity is inversely proportional to the amount of cortisol present in the sample.

Method. This assay is based on a protocol designed by Salimetrics LLC (USA). All work is done with the assistance of a Tecan Freedom 150/8 or an Evo 2 liquid handler. After preparation, 25µl of sample, standards (to give an accurate assay range), or controls (to give a zero or saturated reading) is added to the appropriate well in duplicate. Following this, 200µl of 1:1600 diluted conjugate solution is added to the well. The plate is then shaken for 5 minutes and heated to room temperature for an additional 55 minutes. The plate is then washed 4 times in wash buffer using either a Tecan Columbus or Hydroflex plate washer. A tetramethylbenzidine (TMB) substrate (200µl) is then added to each well before the plate is again shaken for 5 minutes at 500rpm and the heated to room temperature in a light-controlled (i.e. dark) environment for a further 25 minutes. After this, 50µl stop solution (1M sulphuric and 8M acetic acid) is introduced to the well. Plates are shaken for a further 3 minutes at 500rpm before being read at 450nm using an Infinite or Sunrise plate reader.

2.4 Alpha Amylase Assay

Principle. This assay is used for the kinetic measurement of sAA. The method employs the use of the substrate 2-chloro-p-nitrophenol, which is linked with maltotriose (Pointe Scientific). Together, these react with sAA resulting in a yellow coloured product that can be measured spectophotometrically. The rate of this reaction is directly proportional to the amount of sAA present, thereby producing a way of quantifying the enzyme.

Method. This assay is completed using a robotic assistance to pipette saliva samples and diluent into the well, but the second stage is manual with the aid of a multi-channel pipette. To start, via robotic aid, samples are diluted to 1:200 ratio by first diluting 1:10, then 1:10 again. Following dilution, 8µl diluted saliva sample (or control) is added to the

appropriate wells of a microtitre plate. Manually, 320µl preheated (to 45°C) substrate is then added to each well using a 1ml multichannel pipette[1]. The plate is then shaken immediately whilst being heated to 37°C before the optical density is read at 405nm using an Infinite or Sunrise plate reader exactly one minute after the substrate was added. The plate is shaken and incubated as before, before being read a final time at the three minute marker. The difference between the two readings is then multiplied by a conversion factor to account for the dilution phase, resulting in a measure of sAA (U/ml).

2.5 Flow Rate

Flow rate is measured in terms of ml/minute, and can be measured gravimetrically by assuming 1ml saliva weighs 1g (Chicharro, Lucia, Perez, Vacquero, & Urena, 1998). Dividing the delta of the sample tubes (pre- and post-sample) by the number of minutes the sample was taken over gives a ml/minute calculation. Flow rate can be multiplied by analyte concentration measures (e.g. sAA) to give a measure of analyte output over time (secretion).

2.6 Storage and Destruction of Samples

After assaying, samples are re-frozen at -80°C until all analysis has been completed after which they are disposed of. Samples undergo centrifugation each time they are thawed for the purposes of assaying. Every effort is made to keep the amount of freeze-thaw cycles to a minimum in order to preserve the sample quality as advised by Granger, Swartz, Booth, Curran, and Zakaria (1999). Once analysis is complete, samples are autoclaved at 131°C for purposes of sterilisation before being incinerated.

[1] This is a crucial part of the assay owing to its acute time-sensitive nature. The multichannel pipette is capable of aspirating enough substrate to dispense over three columns per time, which enables rapid coverage of the plate.

3.0 CHAPTER THREE: STUDY ONE

On being rejected: Psychological and physiological responses to an acute social rejection task

Prior to introducing a measure of cognitive bias or attempting to modify cognitive biases within the studies reported in this thesis, it seemed pertinent to firstly establish a reliable stressor that could be used to demonstrate the effects of naturally occurring or modified interpretive or attentional bias on an individual's vulnerability to stress. Therefore, the overall objective of this first study was to develop a reliable laboratory stressor that could be used in the subsequent studies of this thesis.

Since the development of the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993) a general consensus has emerged among researchers in the field for cortisol to be an appropriate biomarker to reflect HPA responses to stress paradigms (e.g. Buchanan, al'Absi, & Lovallo, 1999; Cacioppo et al., 2000; Ellenbogen, Schwartzman, Stewart, & Walker, 2002; Gaab et al., 2002; Pruessner, Hellhammer, & Kirschbaum, 1999). Whilst the literature is laden with studies employing stressor tasks that aim to induce changes in stress-related physiology, there remains a large inconsistency between those that achieve this and those that either fail to observe any difference or in some cases even observe the complete opposite. Dickerson and Kemeny (2004) conducted a meta-analysis of 208 such studies and concluded that task-related increases in cortisol were most prominent and reliable when the stressor included three key elements; where individuals felt they were being judged by others (socio-evaluation), where the participant had little or no control over the situation (uncontrollable), and when participants were motivated to perform well (motivation).

Tasks developed to challenge achievement/academic ability pose an understandably stressful situation. For example, such tasks test an individual's mental capacity and can incorporate elements of failure, which provides an unpleasant sensation. In terms of conforming to Dickerson and Kemeny's (2004) three key principles, these tasks tend to include aspects of uncontrollability (e.g. difficulty of task) and motivation (i.e. not wanting to fail), but do not consistently include a social evaluative element. Alternatively, psychosocial

stressors additionally threaten an individual's sense of belonging and, thus, contain the third socio-evaluative factor. The need to belong has long been stressed as a basic yet essential requirement. Indeed, Maslow (1943) held it among the top five of the most fundamental satisfactions (Kune, 1992), and Baumeister and Leary (1995) claim that regular interpersonal interaction is key to maintaining a healthy emotional and cognitive status. A stressor that features the denial of this sense of belonging amongst society (e.g. social rejection) could be argued to contain all three of Dickerson and Kemeny's (2004) elements. Humans crave social acceptance (motivation), which can be achieved following successful interactions with others. All social interaction likely contains evaluation and uncontrollability; an individual's reaction to us is largely determined by them according to a subjective set of norms and expectations, and is therefore uncontrollable. The present study shall therefore opt to develop a laboratory-based social stressor in an attempt to deliver a reliable psychological and biological stress response.

Tasks that induce the perception of social rejection have proved successful in eliciting robust physiological responses. For example, Stroud, Salovey, and Epel (2002) developed a social rejection stressor that involved participants being gradually excluded from two interactions with confederate researchers through both verbal and non-verbal cues. Stroud et al. found significant increases in cortisol in response to this social rejection task, but only in female participants. Alternatively, male participants produced a significant cortisol response to academic stressors (mental and verbal tasks), to which female participants appeared less (physiologically) affected. Gender is a factor known to influence cortisol response (e.g. Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Kirschbaum, Wust, & Hellhammer, 1992), therefore this pattern of response is not completely unexpected. Indeed, Stroud et al. referred to Taylor et al.'s (2000) tend and befriend hypothesis to account for the observed differences. This theory reasons that females adopt a defence that is more likely to

aid their stereotyped nurturing and social roles, whereas males are motivated by Cannon's (1929) more traditional 'fight or flight' response. Research has since provided empirical support for this theory, with evidence suggesting females demonstrate coping strategies that are more evocative of Taylor et al.'s theory. For example, Turton & Campbell (2005) identified that females were more inclined to cope using strategies associated with the tend and befriend theory (e.g. turn to friends for advice) than through fight or flight (e.g. using aggression). Consequently, females might be more sensitive to interpersonal challenges (e.g. social rejection) while males might respond more to instrumental challenges (e.g. intelligence tests). Aside from these influences of gender, Stroud et al. were successful in demonstrating the effectiveness of a social rejection laboratory stressor.

In keeping with the theme of social rejection, Blackhart, Eckel, and Tice (2007) developed an alternative social rejection task that obviated the need for confederate researchers as was necessary in Stroud et al.'s (2002) study. Blackhart et al.'s task required participants to take part in group (4-6 participants) ice-breaker discussions before being informed they would need to select a partner to work with on a group task. Participants were instructed not to choose anyone they knew or were friends with, and were asked to give two options of partners whom they considered they might work well with. Participants were then divided into individual rooms and, after a short delay, each told that they had to complete the ensuing task alone either because no-one had chosen to work with them (social rejection), everyone had chosen to work with them which could not logistically be managed (social inclusion), or due to an administrative error in assigning them a group (control). Results showed a significant increase in cortisol following social rejection, matched with reduced positive affect and increased negative affect. Interestingly, however, no gender effects were observed in their study.

Given the wealth of literature detailing the difficulties in inducing a physiological stress response using a laboratory stressor (see Dickerson & Kemeny, 2004), the purpose of the current study was to replicate the methodology and findings of Blackhart et al. (2007). Specifically, this study aimed to use a social rejection stressor to produce a robust increase in cortisol, and to investigate whether a reliable stress-induced change in sAA could be evoked using this specific paradigm. However, several modifications were made to Blackhart et al.'s protocol in the current study. First, in Blackhart et al. participants were instructed to not rate people they knew or were familiar with. This might have somewhat stalled rejected participant's responses as they were, in effect, being rejected by people to whom they had no existing connection; a point that the authors themselves note. For this reason, the current study omitted the instruction to only rate participants to whom they had no affiliation in a bid to augment any feelings of rejection. Second, Blackhart et al.'s protocol required each participant to rate just two other participants. In the current study, participants were provided with a space to rate all but one of the other participants in the group. So, for example, a group of 6 participants were asked to rate 4 people in terms of whom they would prefer to work with; thus forcing just one person to remain unrated. This amendment was implemented to intensify negative emotions as working alone through rejection in the current study would imply the participant has remained unrated by every other participant rather than simply not being rated as one of two options. Third, in line with Stroud et al.'s (2002) finding that social stressors were more effective for female participants, the current study was conducted using female participants only regardless of the lack of such findings in Blackhart et al.'s study.

It was hypothesised in the current study that increases in cortisol and sAA, in addition to a worsening of emotional state (e.g. reported stress) would occur following social rejection. Alternatively, social inclusion was hypothesised not to influence cortisol, sAA, or emotional state. In addition to measures of state emotion (e.g. reported happiness, stress,

etc.), trait measures of factors known to influence physiological activity (e.g. personality) were measured. The reasons behind these measures were two-fold. Firstly, comparisons could be made between groups (social rejection, social inclusion) to ensure successful randomisation of potentially influential factors. Second, completion of the questionnaires served to pass time between saliva samples. These measures were not analysed in terms of how they influenced psychophysiological responses to the task, but were analysed for between group differences. No between group differences were hypothesised.

3.1 Method

3.1.1 Design

The study adopted a mixed factorial design with group (social rejection, social inclusion) as a between subjects factor and time point (5 measures) as a within subjects factor (see Figure 1). Time points were 15 minutes into the study (baseline 1), 25 minutes into the study immediately before the social manipulation (baseline 2), and 10, 20, and 30 minutes after the social manipulation (SM +10, SM +20, and SM +30, respectively). At each time point, saliva samples (dependent variable) were collected to access physiological reactivity to the social manipulation. Self-reported measures of mood (dependent variables: reported stress, optimism, happiness, tenseness, and distress) were also taken at each time point. Measures of chronic depression, stress, trait anxiety, personality and interpersonal support were taken once during the study to assess participant characteristics and potential influences on stress vulnerability.

3.1.2 Participants

Ninety nine female undergraduates from Anglia Ruskin University expressed an interest in the study and were screened using the Spielberger Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). Of these, 73 responders who scored below 50 on the scale were formally invited to take part in the study[2]. Thirty nine participants[3] between the ages of 18 and 42 years old (M = 22.12, SD = 4.09) accepted the

[2] This screening procedure was implemented as required by Anglia Ruskin University's Faculty Research Ethics Panel as a precaution to prevent people with clinical levels of anxiety from being included in the study.

[3] A power calculation was initially conducted using the conservative assumpion of a small effect size (d = .25), which determined that optimal statistical power (.95) would be achieved with 64 participants in each condition (G*Power 3; Faul, Erdfelder, Lang, & Buchner, 2007). For practical reasons it was not possible to recruit such large sample sizes for all the studies contained within this thesis, given time and financial constraints. Whilst the power is not ideal, sample sizes in the studies contained within this thesis are comparable to those in the published literature (e.g. Dandeneau, Baldwin, Baccus, Sakellaropoulo & Pruessner, 2007; MacLeod, Rutherford, Campbell, Ebsworthy, & Holker, 2002; Stroud, Salovey, & Epel, 2002). It is recognised that recruiting a smaller sample increases the risk of incorrectly rejecting the experimental hypothesis.

invitation and were randomly assigned to one of two conditions: social rejection (age *M* = 22.78, *SD* = 5.15) or social acceptance (age *M* = 21.43, *SD* = 2.50). As a group, state anxiety averaged 42.91 (*SD* = 9.53), with socially rejected participants averaging 41.88 (*SD* = 9.76) and socially included participants averaging 43.88 (*SD* = 9.51).

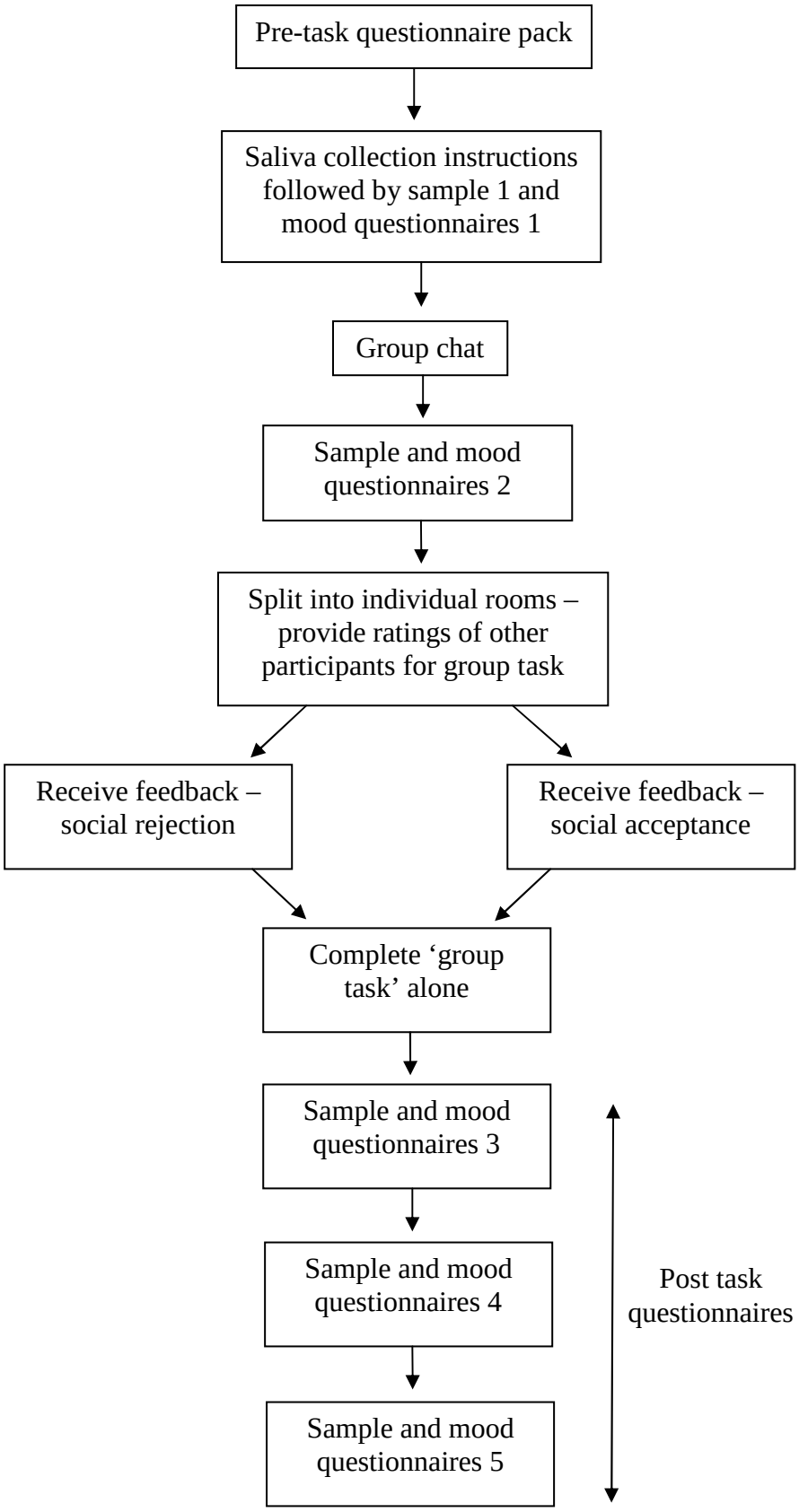

Figure 1. Overview of Study 1's experimental design

3.1.3 Materials

Psychological measures. Prior to giving their first saliva sample, participants completed a questionnaire asking about several aspects of compliance (e.g. when they last ate or drank) to confirm adherence to instructions given relating to the hours leading up to participating in the study. Participants were also asked questions relating to their health behaviour (e.g. how much alcohol they had consumed in the previous week), their oral and overall health and details regarding any medication they were currently taking. This was to collect background data that could be used with retrospect to help identify and justify outliers.

Stress-arousal checklist. Each time participants gave a saliva sample, they completed a copy of the Stress-Arousal Checklist (SACL; Mackay, Cox, Burrows, & Lazzerini, 1978), which is based on a two-dimensional model of mood. One dimension (stress) focuses on feelings of pleasantness or unpleasantness, while the other dimension (arousal) is based on feelings of alertness or drowsiness. The 34-item scale includes both positive and negative mood-describing adjectives that focus either on stress (18 items) or arousal (14 items). For example, “cheerful” (positive stress item), “tense” (negative stress item), “energetic” (positive arousal item), and “sluggish” (negative arousal item). For each item, individuals must select a response on a four-point scale ranging from 1 (*definitely*) to 4 (*not at all*) according to how accurately the adjective describes how they are feeling at that specific moment in time. Items that apply to the individual (where the adjective has received a score of 1 (*definitely*) or 2 (*slightly*)) are recoded as a 1, while items that do not apply to the individual (those that have received a score of 3 (*unsure*) or 4 (*not at all*)) are recoded as a 0. Overall stress scores are calculated by totalling the recoded positive stress items that have received a score of 1 (e.g. where an individual has said they are feeling slightly or definitely tense) with the recoded negative stress items that have received a score of 0 (e.g. where an

individual has said they are unsure or do not feel cheerful). Overall arousal scores are calculated in the same way using the relevant positive and negative arousal items. Cronbach's alpha has been typically reported as between .80-.90 for both scales, with the stress scale invariably being slightly higher (e.g. Lau & Morse, 2005; O'Connor, Cobb, & O'Connor, 2003)[4].

Visual analogue scales. After completing each SACL, participants completed four visual analogue scales (VAS; Aitken, 1969; Bond & Lader, 1974) to assess fluctuations in mood over time during the study. Each of these VAS consisted of a 10cm line, with terminals labelled *pessimistic* to *optimistic*, *depressed* to *happy*, *distressed* to *not distressed*, and *tense* to *relaxed*. Participants were asked to place a cross along the line according to how they felt at that moment, which was converted to a score out of 100 by multiplying the length along the line (in cm) by 10.

Post-task questionnaire battery. *Depression Anxiety Stress Scale (DASS).* The DASS (Lovibond & Lovibond, 1995a; 1995b) was developed to provide a greater understanding of the emotions and underlying constructs of the terms generally described as depression (e.g. pessimistic, lacking in motivation), anxiety (e.g. panicky, awareness of a pounding heart) and stress (e.g. easily irritable, intolerant of change). Internal consistency for all three subscales is high (Cronbach's alpha = .88, .82, and .90, respectively) with a total scale α consistency of .93 (Henry & Crawford, 2005). Though originally a 42-item scale, a shortened version containing 21 items is commonly used in research with 7 items dedicated to each of the three subscales. Items apply to experiences over the previous week, for example "I felt that life was meaningless" (depression), "I felt I was close to panic" (anxiety), and "I tended to over-react to situations" (stress). Individuals are required to rate the extent to which each statement was

[4] This scale has been used here and in future studies contained within this thesis to measure stress, therefore only the stress dimension is considered in analysis.

relevant to them on a four point scale ranging from 0 (*Did not apply to me at all*), to 3 (*Applied to me very much, or most of the time*). Scores can be determined for each subscale by summing the responses for each item. Alternatively a total score can be attained by summing the subscales, which provides an indicator of general negative symptomology.

General Health Questionnaire (GHQ). The GHQ (Goldberg, 1972; Goldberg & Williams, 1988) is a popular measure of psychological distress used in research and assesses participants on four dimensions of mental wellbeing; depression, anxiety, somatic symptoms and social withdrawal. Although the GHQ is available as a 12-item, 28-item, 30-item or 60-item, the GHQ-28 is most commonly used and, hence, was used in this study. GHQ scores frequently correlate highly with additional measures of psychological distress or well-being (Jackson, 2007) suggesting concurrent validity. Internal consistency is good both for the whole scale (Cronbach's α =.90) or individual subscales (between .71 - .85; Vallejo, Jordán, Díaz, Comeche, & Ortega, 2007). The GHQ applies to a person's experiences over the past few weeks. Examples of items on the GHQ-28 scale include "Have you recently lost much sleep over worry?", and "Have you recently been satisfied with the way you've carried out your tasks?". Items are resolved by four possible answers, ranging from 0 (*not at all)* to 3 (*much more than usual*) (or those equivalent). Responses can be summed to give totals for the four subscales or an aggregate total.

Perceived Stress Scale (PSS). The PSS (Cohen, Kamarck, & Mermelstein, 1983) is available as a 4-item, 10-item, or 14-item scale and is used to give a measure of an individual's perception of stress over the preceding one month. In the current study the 10-item scale was used, as this version is considered to have superior sensitivity to psychometric distinction compared to the alternate versions (e.g. Cohen & Williamson, 1988; Lesage, Berjot, & Deschamps, 2012). The scale was designed to assess how uncontrollable, unpredictable and overloading an individual considers their life to be. Internal consistency for

the 4-item version is relatively low at Cronbach alpha = .60, while the 10-item version has been reported to have adequate consistency (Cronbach alpha = .78) (Cohen & Williamson, 1988). Individuals are required to rate how often they have felt a certain way. For example, "In the last month, how often have you felt you were effectively coping with important changes that were occurring in your life?" or "In the last month, how often have you felt that difficulties were piling up so high that you could not overcome them?". Responses are scored from 0 (*never*) to 4 (*very often*). Scores on the 10-item scale range from 0 to 40, and are obtained by reversing the seven positive items (i.e. where a high score indicates a desirable option) and summing the ratings.

Personality Inventory. Due to findings of a relationship between personality and cortisol reactions to stressors (e.g. Oswald et al., 2006), personality was included as a trait measure. The personality inventory used was taken from the International Personality Item Pool (IPIP; Goldberg et al., 2006). Similar to the Costa and McCrae's (1992) five factor model, the personality inventory measures five independent subscales, namely extraversion (e.g. "I talk to a lot of different people at parties"), agreeableness (e.g. "I sympathise with others' feelings"), conscientiousness (e.g. "I am exacting in my work"), emotional stability (e.g. "I am relaxed most of the time"), and intellect (e.g. "I am quick to understand things"). The five subscales each have good internal reliability, with Cronbach's alpha coefficients of .87, .82, .79, .86, and .84, respectively. Each subscale consists of 10 items including positive and negative phrases that individuals have to rate according to how accurately the statements reflect their own behaviour on a five-point scale ranging from 1 (*very inaccurate*) to 5 (*very accurate*). Negative items (e.g. for extraversion, the item "I have little to say") are reverse scored before a total for each of the five subscales can be attained by summing the relevant responses.

Spielberger Trait Anxiety Inventory (STAI-T). Described as the "...definitive instrument for measuring anxiety in adults..." (Mind Garden, 2010, "State-Trait Anxiety Inventory for Adults", para. 1), the STAI (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) is divided into items measuring transitory anxiety (state) and more stable chronic anxiety (trait). Each subscale has 20 dedicated items that are measured on a four point Likert scale ranging (on the trait items) from 1 (*almost never*) to 4 (*almost always*). Examples of items measuring trait anxiety include, "I [generally] feel inadequate", and "I am [generally] a steady person". State anxiety or trait anxiety scores can be calculated by summing participant responses, with some items needing to be reverse scored. The scale has been found to share high positive correlations (>.70) to other anxiety scales, for example, the Anxiety Scale Questionnaire and Manifest Anxiety Scale (Spielberger, Reheiser, Ritterband, Sydeman, & Unger, 1995). Spielberger, Gorsuch, and Lurshene (1970) also demonstrated good test-retest reliability for both subscales (trait: r = .76 for females, r =.84 for males; state: r = .92 for females, r = .83 for males), although there is a general acceptance that state anxiety scores tend to be slightly higher at second measurement.

Interpersonal Support Evaluation List (ISEL). The ISEL (Cohen & Hoberman, 1983) was designed to measure the perceived availability of social support, which has been posited to act as a form of protection to stress-induced pathology when perceived stress is high. The scale measures four independent subscales, namely tangible, belonging, self-esteem, and appraisal, and can generate a broad overall rating of potential social resources available. The 40-item scale is made up of an equal number of positive statements such as "There are several people that I trust to help solve my problems", and negative statements such as "In general, people do not have much confidence in me". Individuals are required to rate how relevant each statement is to them on a four-point scale ranging from 0 (*definitely false*) to 3 (*definitely true*). Scores for negative items are reversed before the scores are summed to give

either an overall total or subscale total value, where a high score indicates a high level of potential social support. The ISEL demonstrates good reliability, with test-retest reportings of .87 (r = .71-.87 for the individual subscales) and internal consistency α ranging from .77-.86 (Cohen & Hoberman, 1983).

Stressor

Stress was manipulated by attempting to induce feelings of either social rejection (stress group) or social inclusion (comparison group). Prior to the induction of these feelings, participants chatted with each other informally for approximately 5 minutes. Participants were informed that this period was designed to relax them and to enable them to get an idea of who they might prefer to work with later in the study. Participants were then divided into individual rooms and asked to select a partner for an upcoming group exercise by providing ratings of their fellow participants. Ratings were instructed to be based on who they would prefer to work with and who might best respect and fairly consider their opinion. Space was provided on the rating sheet for participants to rate all but one of their fellow participants.

After collecting the slips, the researcher entered each individual room and informed the participant that there had been a problem with the group allocation. Participants were either informed that every person had requested to work with them as their primary preferred option, therefore, they could not be fairly assigned a group (social inclusion), or that they remained unrated and so could not be assigned a group (social rejection). Regardless of their condition, all participants completed the ensuing ‘group’ task alone; the difference being in whether they were led to believe this was due to them being too popular or too unpopular to be assigned a group.

“Group Task”

Participants were instructed to complete a group exercise which they were informed was designed to investigate how group dynamics interact with individual mood and physiology. This task was actually a filler task, completed (alone) once people had been socially rejected or accepted in order to uphold the illusion and allow participants to ruminate on their respective social manipulation. The task was designed and delivered using Microsoft PowerPoint software. Photographic headshots of males and females were individually presented in the middle of a computer screen for six seconds each. A coloured screen was then displayed for a further five seconds, during which participants were instructed to make a “group” decision indicating on a Likert scale how friendly they thought the person was likely to be based on the photo alone. A total of 48 photos were presented in this sequence.

3.1.4 Saliva Collection and Analysis

Saliva was collected five times over the duration of the study via a passive drool technique into a 2ml cryovial tube (Greiner Bio-One Ltd, UK). For this, participants were instructed to clear their mouths by swallowing, then position their head forward with their chin tilted towards their chest for a 90-second period to allow saliva to accumulate at the front of their mouths. This was then transferred to the appropriate, individually labelled, cryovial tube with the assistance of a short section of straw. This procedure was repeated into the same tube to constitute one sample.

The first two collections were directed and timed by the researcher. The instructions and timing for the final three collections was inbuilt into the group task programme. After the last photo presentation, the task instructed the participant to find the corresponding cryovial for the third saliva sample (the instructions were designed for a group audience). Upon clicking the mouse, a 90-second period was timed for the first half of a sample. This was

followed by a screen asking the participant to deposit the saliva into the tube before clicking the mouse again to time a further 90-second period. The following screens were timed to enable the fourth and fifth samples to be taken at 10 minute intervals.

Samples were frozen at -80°C until required for assaying. Samples were analysed for levels of cortisol and sAA. More detailed information on these procedures can be found elsewhere (see Chapter two).

3.1.5 Procedure

Ethical approval was obtained from Anglia Ruskin University Faculty Research Ethics Subcommittee. Participants were instructed not to eat, drink (other than water), or smoke for 30 minutes prior to the study, and to refrain from vigorous exercise for 90 minutes preceding the study. Participants in groups of 3-5 were met by the researcher in a lecture room. They were issued with a participant information sheet and given time to read it and ask questions prior to signing a study consent form. Participants were asked to drink a cup of mineral water to rinse their mouths of any food debris. Following this, participants completed a pre-task questionnaire which included questions relating to health behaviour, oral hygiene, and general health. Ten minutes after taking the drink, participants were issued with instructions on how to give a saliva sample based on the passive drool method described above. Participants then gave their first saliva sample, after which they completed the SACL and four VAS based on their current feelings and emotions. Participants were then encouraged to chat freely as a group without the presence of the researcher (once a dialogue was established).

Ten minutes after their first sample, participants provided their second saliva sample using the same procedure as before. Again, at this point, they completed some questionnaires (SACL and the four VAS). Participants were then given instructions on how the group task

would commence, that they were to be presented with still images of faces which they were to rate in their group by selecting a number on a Likert scale depending on how kind (or mean) they thought that person was likely to be based on the still image alone. They were told that each member of the group was to ensure they had the same rating. Participants were then instructed how to choose their groups before being separated into individual rooms. Participants were required to provide ratings of their peers according to who they would prefer to work with and were then issued with information concurrent with their condition (see *Stressor* section above).

Participants remained in their individual rooms for the remainder of the session. After completing the "group" task (ten minutes after the social manipulation), participants gave a third saliva sample and completed a third SACL and four VAS. They were then instructed to start completing the post-task questionnaire battery, which was designed to measure aspects of their personality, general and perceived health, and interpersonal support. Ten minutes after the third sample, participants were instructed to give their fourth sample and complete a fourth SACL and four VAS. They then returned to the questionnaire battery for a period of ten minutes before being asked to give their fifth and final saliva sample, and complete their final SACL and four VAS.

Participants were given debrief sheets in their individual rooms, explaining the underlying nature of the study, before being debriefed as a group in the same room as they started. The debrief was conducted in this manner to prevent any unnecessary embarrassment from returning to a group they might have believed had recently rejected them. Once questions and concerns had been addressed, participants were paid £8 to compensate them for their time.

3.1.6 Data Analysis Plan

Prior to testing the study hypothesis, the data was explored to ensure it met the assumptions of parametric testing. Data from trait questionnaires was also explored briefly to check for potential group differences. To test the study hypothesis regarding changes over time and influence of social manipulation, a series of repeated measures ANOVAs were conducted on the relevant dependant variables (e.g. reported stress, cortisol, etc), with time as a within subjects factor and group as the between subjects factor. Main effects of time are reported though not necessarily explored where they are qualified by time x group interactions. For ease of clarity, group main effects are largely not reported unless significant or relevant to the point of note. Where appropriate, a-priori and post-hoc testing was carried out via paired *t*-tests to isolate significant interactions. Corrected alpha levels (Bonferroni) were calculated and are reported.

3.2 Results

3.2.1 Data Exploration

All data was explored for outliers and to check the data met the assumptions for parametric testing. All data obtained by saliva analysis (sAA activity, sAA secretion, cortisol concentration and flow rate) included several outliers and showed positive skewing, and was therefore log transformed, which successfully normalised the distribution (Nicolson, 2008). All analyses were conducted using logged data, however descriptive and graphical representation of the means and measures of variation are presented using unlogged data.

A series of 2 (group; reject, accept) x 2 (time; baseline, baseline 2) repeated measures ANOVAs were conducted in the initial analysis of the data and revealed an apparent difference in the physiological data between the first two samples; baseline 1 and baseline 2 (taken approximately 10 minutes apart). Looking more specifically at these two sample points, flow rate was found to significantly increase, $F(1, 37) = 10.53$, $p = .002$, $\eta_p^2 = .22$, from an average of .24mls/min ($SD = .15$) to an average of .32mls/min ($SD = .16$). There was no main effect of time on cortisol concentrations, $F(1, 34) = .02$, $p = .89$, $\eta_p^2 < .001$, while sAA activity, $F(1, 29) = 12.00$, $p = .002$, $\eta_p^2 = .29$, and secretion, $F(1, 29) = 35.40$, $p < .001$, $\eta_p^2 = .55$, both considerably increased. No significant group main effects or time x group interactions were observed for any of the above findings (all F values < 1). For this reason, future analyses are conducted using the second of the two samples as a baseline measure; hereafter referred to as baseline 2.

3.2.2 Participant Characteristics

There were no significant differences between the two groups according to mean self-reported levels of trait depression, stress, trait anxiety or any of the personality subscales (see

Table 1). Participants in the social inclusion group reported having significantly more overall functional interpersonal support compared to socially rejected participants. Broken down into the four subscales, perceived self esteem and appraisal showed no significant differences between the two groups, while a sense of belonging and tangible support were found to approach significance.

3.2.3 Self-Reported Stress

Exploring the hypothesis that social rejection would lead to an acute increase in stress, a 2 (condition; rejection, inclusion) x 3 (time; baseline 2, 10 minutes after social manipulation, 20 minutes after social manipulation) repeated measures ANOVA was conducted on the self-report stress data from the SACL. There was a significant time main effect, $F(1.67, 61.78) = 5.44$, $p = .01$, $\eta_p^2 = 0.13$ (Greenhouse-Geisser reporting). Further investigation identified a significant rise in reported stress between baseline 2 and 10 minutes after the social manipulation, regardless of participants' condition, $t(38) = -2.85$, $p = .01$, $d = .41$ (Bonferroni corrected alpha = 0.025). As can be seen from Table 2, participants do appear to start recovering from this increase in stress, although the difference (decrease between 10 and 20 minutes after SM) remained just above the corrected level of significance, $t(38) = 2.23$, $p = .03$, $d = .29$.

Table 1

Participant characteristics

		Social rejection		Social inclusion		
Questionnaire	**Factor / Subscale**	**Mean**	**SD**	**Mean**	**SD**	***p* value**
STAI	*Trait anxiety*	41.64	10.65	44.38	9.60	.55
DASS	*Depression*	5.45	7.22	5.06	4.81	.78
	Anxiety	5.36	4.61	4.06	2.86	.56
	Stress	7.27	6.40	7.94	4.37	.93
	Total	36.18	34.62	34.13	21.31	.80
GHQ	*Distress*	98.18	27.13	99.81	22.96	.65
PSS – 10	*Stress*	18.55	4.87	18.31	6.60	.99
Personality	*Extraversion*	31.45	1.21	26.81	3.05	.16
	Emotional stability	28.64	3.14	26.81	3.39	.30
	Conscientiousness	33.64	3.17	32.25	3.13	.12
	Agreeableness	33.27	4.24	33.50	2.92	.77
	Intellect / Openness	34.09	3.65	33.00	4.40	.66
ISEL	*Appraisal*	23.13	6.83	26.33	3.90	.10
	Tangible	21.53	5.14	24.44	3.78	.05
	Self esteem	19.53	5.28	20.78	3.95	.35
	Belonging	21.47	5.88	24.83	4.59	.08
	Total	85.67	20.59	96.39	14.08	.02

Note: STAI – State trait anxiety inventory, DASS = Depression Anxiety Stress Scale, GHQ = General Health Questionnaire, PSS = Perceived Stress Scale, ISEL = Interpersonal Support Evaluation List.

Table 2

Total reported stress over time

	Mean Reported Stress *(N = 39)*	*SD*
Baseline 2	3.00	4.01
Social manipulation + 10 minutes	4.64	3.92
Social manipulation + 20 minutes	3.38	4.62

Mean stress scores did appear to show a higher peak in response to social rejection than to social inclusion (see Figure 2). However, contrary to the hypothesis, the time x group interaction was not found to be statistically significant, $F(1.67, 61.78) = 0.83$, $p = .42$, $\eta_p^2 = .02$.

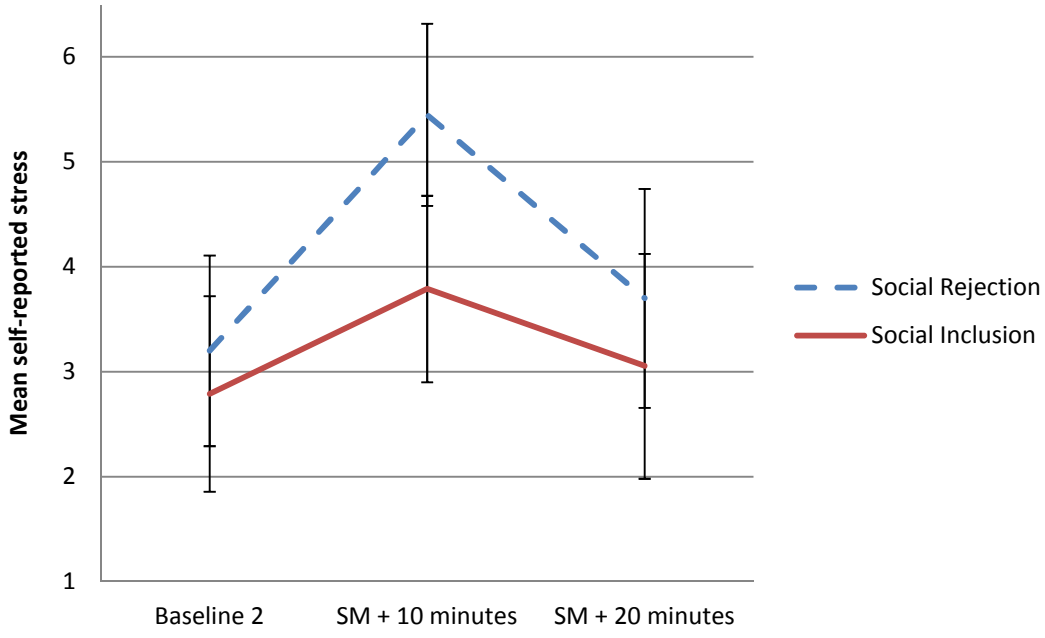

Figure 2. Mean stress scores and variation (SE). SM = Social manipulation.

3.2.4 VAS

A 2 (condition; rejection, inclusion) x 3 (time; baseline 2, 10 minutes post-social manipulation, 20 minutes post-social manipulation) repeated measures ANOVA was conducted on each of the VAS to assess the acute effects of the social manipulation. No significant main effect of time on reported optimism was found, $F(2, 72) = .30$, $p = .74$, $\eta_p^2 = 0.01$, though a significant time x condition interaction was found, $F(2, 72) = 5.58$, $p = .01$, $\eta_p^2 = 0.13$. Upon further investigation, participants who were socially rejected were found to report significantly less optimism immediately after the social manipulation, $t(19) = 2.88$, $p = .01$, $d = .31$ (Bonferroni corrected $\alpha = 0.0125$), whereas participants who were socially included showed no change in reported optimism before or after the social manipulation, $t(18) = -1.44$, $p = .17$, $d = .18$ (see Figure 3a). No significant differences were found in self-reported levels of optimism from 10 to 20 minutes after the social manipulation for either group (social rejection $p = .43$; social inclusion $p = .89$).

For self-reported levels of happiness (see Figure 3b) a significant main effect of time was revealed, $F(1.68, 60.29) = 3.68$, $p = .04$, $\eta_p^2 = 0.09$, with a trend time x group interaction also emerging suggesting different levels of happiness according to whether participants had been socially rejected or included, $F(1.68, 60.29) = 2.81$, $p = .08$, $\eta_p^2 = 0.07$. Post-hoc analysis of the main effect of time illustrated a significant decrease in levels of self-reported happiness for all participants following the social manipulation phase, $t(38) = 2.60$, $p = .01$, $d = .29$ (Bonferroni corrected $\alpha = 0.03$). There was no change in self-reported happiness from 10 to 20 minutes after the social manipulation ($p = .09$). A-priori investigations of the trend interaction identified a decrease in self-reported happiness immediately following social rejection that fell just short of the revised alpha level, $t(19) = 2.53$, $p = .02$, $d = .49$ (Bonferroni corrected $\alpha = 0.01$), from an average reporting of 71.75% ($SD = 24.02$) to 58.75% ($SD = 28.46$). There was no significant difference in reported happiness from 10 to

20 minutes after the social manipulation, $t(19) = -1.64$, p = .12, $d = .15$. Participants who had been socially included showed no significant change in their reported levels of happiness (baseline 2 – SM + 10 minutes $p = .35$; SM + 10 minutes – SM + 20 minutes $p = .45$).

No significant main effect or interaction was identified for self-reported levels of distress (all p values > .24; see Figure 3c). A significant time main effect was found for self-reported tension, $F(2, 72) = 3.43$, $p = .04$, $\eta_p^2 = 0.09$, though the time x group interaction was not significant, $F(2, 72) = 0.50$, $p = .61$, $\eta_p^2 = 0.01$. Further investigation of the main effect, using a Bonferroni corrected alpha (0.025), found no significant change in overall reported tension immediately after the social manipulation ($p = .47$) but a significant increased relaxed state 20 minutes after the social manipulation, $t(37) = -2.89$, $p = .01$, $d = .25$ (see Figure 3d).

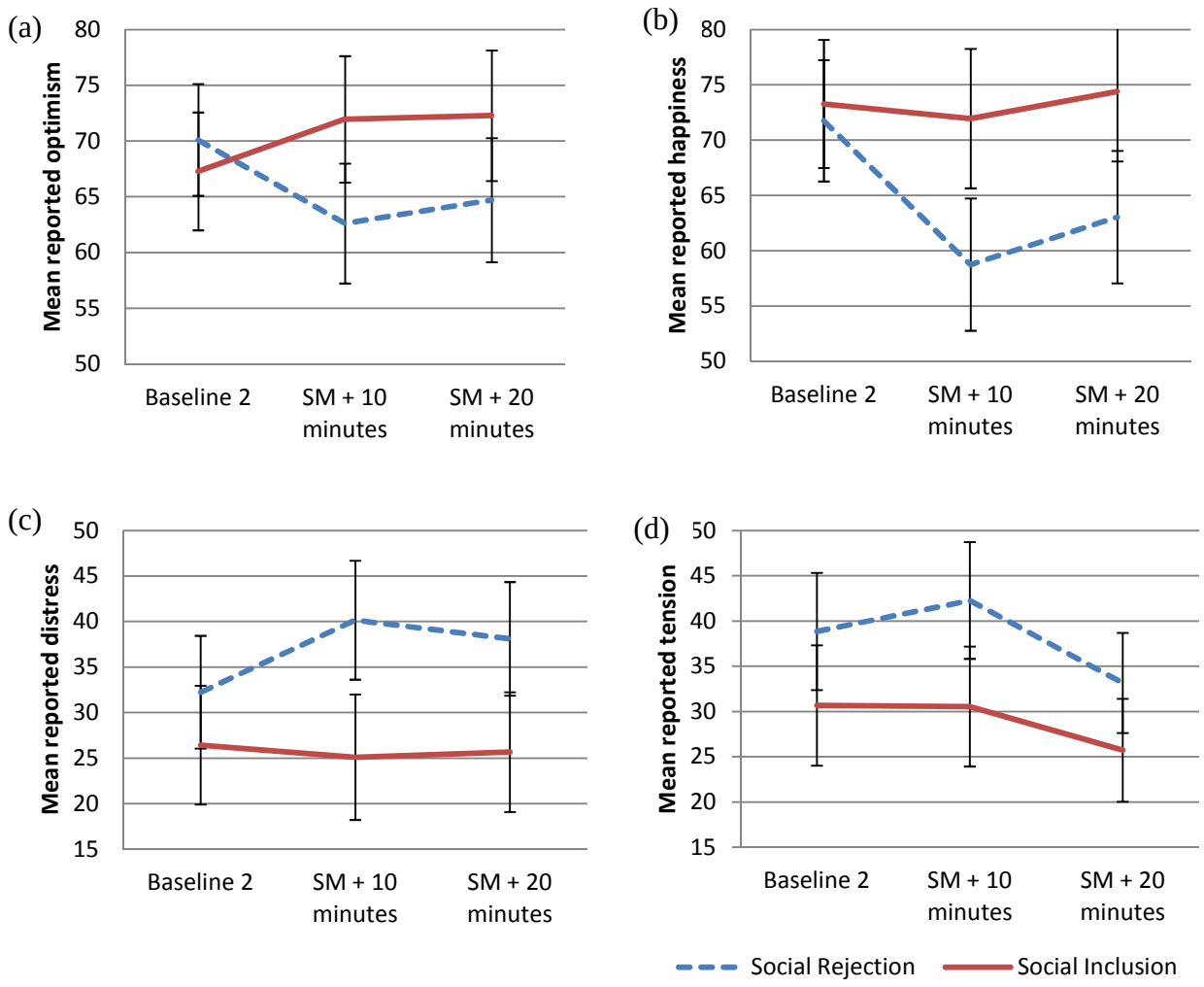

Figure 3. Mean mood self-ratings (with standard error) for optimism (a), happiness (b), distress (c), and tension (d). A higher score indicates more intense feelings of the measure

Summary of Psychological Response

Contrary to the hypothesis, stress was found to significantly increase following the social manipulation in all participants. As expected, for the social rejection group, self-reported optimism and levels of happiness were both found to decrease following the social manipulation task. For the social inclusion group self-reported optimism and happiness did not differ significantly pre- and post- social manipulation. There was no significant variation in reported levels of distress in either condition, which opposes the hypothesis. Furthermore, levels of tension did not differ significantly pre- and post-social manipulation but were

significantly lower 20 minutes post-manipulation than 10 minutes post-manipulation. Overall these findings do not support the hypothesis that social rejection alone would lead to a significantly more negative psychological state, as only the VAS measures of optimism and happiness show changes in the expected manner.

3.2.5 Cortisol

A 2 (condition; rejection, inclusion) x 4 (time; baseline 2, 10, 20, and 30 minutes after the social manipulation) repeated measures ANOVA was conducted to determine the influence of social manipulation on cortisol reactivity. A significant main effect of time, $F(3, 93) = 21.39$, $p < .001$, $\eta_p^2 = .41$, and a significant time x group interaction was found, $F(3, 93) = 3.43$, $p = .02$, $\eta_p^2 = .10$ (see Table 3).

Table 3

Mean cortisol data (µg/dl)

		Baseline 2	SM + 10 minutes	SM + 20 minutes	SM + 30 minutes
Social rejection	Mean	0.10	0.09	0.08	0.07
	SE	*0.06*	*0.05*	*0.05*	*0.04*
Social inclusion	Mean	0.11	0.09	0.09	0.09
	SE	*0.06*	*0.04*	*0.04*	*0.03*

Further investigation of the significant interaction revealed a general pattern of decreasing cortisol concentration for socially rejected participants. Specifically there was a significant decrease between baseline 2 and 10 minutes after the social manipulation, $t(19) = 2.71$, $p = .014$, $d = .20$ (Bonferroni correct $\alpha = .017$). There was also a trend decrease between 10 – 20, $t(18) = 1.96$, $p = .07$, $d = .21$, and 20 – 30, $t(17) = 1.97$, $p = .07$, $d = 21$, minutes after the social manipulation. Additionally, when comparing the difference between the first sample (baseline 2) and final sample (SM + 30 mins), it was found that there was a

significant decrease in cortisol levels in this group, $t(18) = 5.61$, $p < .001$, $d = .59$ (see Figure 4). In contrast, for the socially included group, this decrease appeared evident initially between the baseline 2 and SM + 10 mins samples, $t(15) = 3.96$, $p = .001$, $d = .39$, and then there was no significant change between either 10 – 20 minutes or 20 – 30 minutes after the social inclusion ($p = .56$, $p = .72$ respectively).

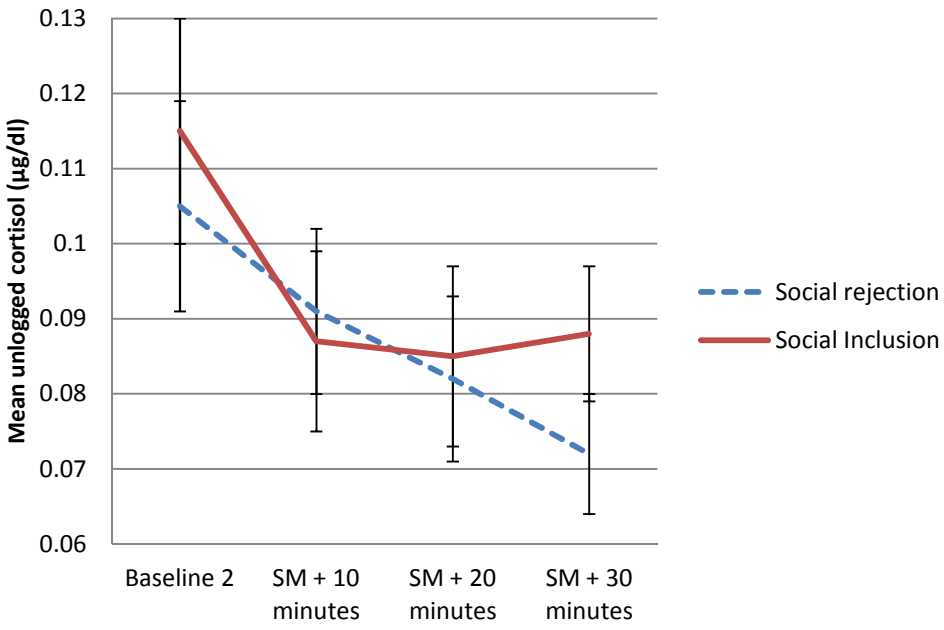

Figure 4. Time x condition group interaction on mean cortisol concentration

3.2.6 Alpha Amylase and Flow Rate

A 2 (condition; rejection, inclusion) x 3 (time; baseline 2, and 10 and 20 minutes after the social manipulation) repeated measures ANOVA was conducted to determine any acute effect of SM on sAA activity. A significant main effect of time was found, $F(1.66, 51.44) = 6.82$, $p < .001$ $\eta_p^2 = 0.18$, however there was no significant time x group interaction, $F(1.66, 51.44) = .27$, $p = .77$ $\eta_p^2 = 0.01$. Upon further investigation of the time main effect, sAA

activity significantly decreased between baseline 2 and 10 minutes after the SM, $t(33) = 3.09$, $p < .001$, $d = .39$ (Bonferroni corrected $\alpha = 0.025$). There was no significant change in sAA activity from 10 to 20 minutes after the social manipulation, $t(34) = -.64$, $p = .53$, $d = .05$ (see Table 4).

In keeping with current advice (e.g. Salimetrics, 2012) when measuring biomarkers that are potentially dependent on saliva flow, sAA secretion (output over time) and saliva flow were calculated and were analysed in the same manner as sAA activity. Interestingly, for sAA secretion rate, there was no significant main effect of time, $F(1.53, 47.43) = .73$, $p = .43$, $\eta_p^2 = 0.03$. Social manipulation was also not found to significantly interact with sAA secretion over time, $F(1.53, 47.43) = .13$, $p = .83$, $\eta_p^2 = 0.19$ (see Table 4).

Table 4

Mean sAA activity and secretion

		Baseline 2	SM + 10 minutes	SM + 20 minutes
Activity (U/ml)	Mean	75.22	55.82	57.85
	SD	*59.70*	*50.20*	*54.37*
Secretion (U/min)	Mean	24.51	21.37	21.95
	SD	*20.67*	*21.49*	*18.63*

Note. SM = Social manipulation.

For flow rate, a significant time main effect was found, $F(1.52, 54.54) = 8.62$, $p < .001$, $\eta_p^2 = 0.19$. No significant time x group interaction was identified, $F(1.52, 54.54) = 2.76$, $p = .09$, $\eta_p^2 = 0.07$. Post-hoc testing of the main effect revealed that saliva flow appeared to follow the exact opposite pattern as sAA activity, with a significant increase between baseline 2 and 10 minutes post SM, $t(38) = -2.68$, $p = .01$, $d = .36$ (Bonferroni

corrected $\alpha = 0.025$), which was maintained at 20 minutes post-social manipulation, $t(37) = -.07$, $p = .95$, $d = .01$ (see Table 5).

Table 5

The relationship between sAA and saliva flow changes over time

	TIMEPOINT COMPARISON	
	Baseline – SM + 10 minutes	SM + 10 minutes – SM + 20 minutes
sAA activity	$p = .004$ Significant DECREASE	$p = .53$ *No change*
Flow rate	$p = .011$ Significant INCREASE	$p = .95$ *No change*
sAA secretion	$p = .19$ *No change*	$p = .25$ *No change*

Note. SM = Social manipulation

Summary of Physiological Response

Cortisol was found to decrease generally throughout the study, which was more prominent in participants who experienced social rejection. sAA activity appeared to significantly decrease following the social manipulation phase for all participants, while flow rate showed the opposite pattern and significantly increased. There was no change in sAA secretion. None of these findings support the hypothesis for a greater physiological activation in response to social rejection.

3.3 Discussion

This study aimed to establish a stressor task that was successful in eliciting a reliable physiological and psychological response, marked by an increase in reported stress and increases in sAA and cortisol. VAS measured changes in emotion due to the stressor. In line with the hypothesis, social rejection was successful at decreasing reported levels of optimism and happiness. However, there was no effect on reported tension or distress. The SACL measured changes in stress in response to the social manipulation and, in contrast to the hypothesis, reported stress was found to increase in all participants following the social manipulation regardless of condition. Levels of cortisol concentration were found to decrease after social rejection but not social inclusion, thereby failing to support the hypothesis which had predicted an increase in cortisol following social rejection. Whilst there was a significant decrease in sAA activity following social manipulation generally, there was also a significant increase in flow rate in an exactly opposite manner and no change in sAA secretion rate, none of which supported the experimental hypotheses.

According to data collected using VAS, social rejection was partially successful in inducing a negative state. However, it is worth noting that all four measures of mood (optimism, happiness, distress, and tension) shared significant positive correlations at all time points throughout the study (weakest correlation: r (39) = .45, $p < .01$; strongest correlation: r (38) = .92, $p < .001$). Considering that a higher value indicated a more intense feeling of emotion, it is initially surprising to observe a positive relationship between all of these variables when two depict positive mood states (optimism, happiness) and two depict negative mood states (distress, tension). However, these scales are rudimentary in their method of measurement – requiring participants simply to place a cross along a continuum line to indicate their current state – and allow participants to choose their own baseline. For this reason, it is only natural that great variation will be introduced as individuals

systematically endorse higher or lower numbers using this scale. Whilst the validity might be questionable, a within-subjects design might still be able to usefully apply these measures to monitor individual change over time. Alternatively, interpretations drawn in a between-subjects study (such as the present study) would be contaminated by such radical inter-variation and so would be unreliable. Future studies within this thesis are therefore advised to adopt more standardised measures of mood.

The lack of any significant effects of social rejection on reported stress above and beyond that of social inclusion opposes the findings of Blackhart, Eckel, and Tice (2007). These results are surprising given that the methodology was based on what Blackhart et al. claim to be a commonly used protocol. Further, Blackhart et al. applied some stipulations to the rating process preventing participants from rating any person with whom they had some form of social affiliation. They proceeded to note in their discussion that lifting this limitation might lead to stronger effects of social rejection as there would be an increased personal significance of being rejected by people they had an existing relationship with. Therefore, the current study should arguably have intensified any feelings of rejection as there were no such stipulations regarding precisely whom participants could or could not rate. However, it remains possible that such an attempt to further reinforce rejected feelings failed on two instances. Firstly, participants signed up to the study independently, with groups largely consisting of people who were not existing friends (though data was not collected to monitor this). Therefore, whilst the occasional instance of friends appearing to reject friends occurred, to a large extent the ratings were made between unfamiliar people as in Blackhart et al.'s study. Second, in instances where friends did appear to reject friends (in apparent favour of unfamiliar people), it is possible that participants started to see through the deception and so became disengaged rather than feel excluded. As no measure of manipulation scepticism was taken before debriefing participants, it is difficult to know definitively whether participants

were entirely persuaded of their social manipulation condition, though post-debrief conversations failed to highlight any specific area of concern. Future research might try to avoid such limitations by including some check on manipulation impression to assess whether participants were successfully influenced.

Alternatively, the rise in reported stress across both socially rejected and accepted participants could result from the nuances of social anxiety. While traditional models of social anxiety posit that it is driven by a fear of negative social judgement (Clark & Wells, 1995), recent attempts to further understand the concept have additionally considered the role of positive evaluation. For example, Weeks, Heimberg, Rodebaugh, and Norton (2008) have shown high correlations between a fear of positive evaluation and measures of social anxiety. Therefore, in the present study, perhaps simply the reception of social feedback was sufficient in producing an increase in perceived stress in the more socially anxious participants. As a measure of social anxiety or fear of positive/negative evaluation was not taken in this study, such a hypothesis is conjecture at the present time. However, it is worth noting at this point that in spite of a significant increase being observed, reported stress levels remained relatively low throughout the study giving the impression that participants were not particularly stressed by the experience. For this reason the above hypothesis seems unlikely to hold true in this instance though remains an interesting consideration for future studies. In further support of this assumption, the present study only recruited participants who scored below 50 on the STAI; a request made by the ethics committee. Blackhart et al. (2007) included no such limitations. It is likely that, in abiding by ethical stipulations, the present study inadvertently selectively recruited a sample who were less sensitive to anxiety-provoking situations. In future situations where the sample is restricted in such a manner, the impact of social rejection might be more effective if exposure is made in a more public environment, with more of an audience presence. For example, Dickerson, Mycek, and

Zaldivar (2008) found that participants who took part in a stressful task (delivering a speech) in front of a judging audience, where the prospect of social evaluation is apparent, showed a significant increase in cortisol relative to when the speech was given to an empty room.

The current study found a decrease in sAA activity in response to both social rejection and social acceptance. This finding is unexpected given that the current understanding suggests that levels of sAA would be expected to increase in response to an acutely stressful event (e.g. Bosch et al., 1996). However, reported stress was found to increase in response to both manipulations therefore it is possible to present a post-hoc rationalisation of this finding. For example, while there are presently no studies that focus specifically on the effects of social rejection as a type of stressor on sAA, the results do provide partial support for studies focusing on the influence of social stressors on other aspects of the ANS. For example, Gunther Moor, Crone, and van der Molen (2010) present findings on heart-rate variability following social rejection that in part draw a parallel to the patterns of general sAA activity in the current study. Gunther Moor et al. claimed that unexpected rejection specifically serves to increase feelings of being hurt, which disrupts the autonomic balance in favour of parasympathetic control, thereby leading to a decrease in heart rate. In support of this theory, Heilman et al. (2008) also found a decrease in heart rate in children exposed to a social challenge which remained absent when exposure was to a physical challenge. The self-report data in the current study showed an increase in stress resulting from the social manipulation element generally (i.e. regardless of whether this involved social rejection or acceptance). It is possible, then, that the decrease in sAA activity seen generally in all participants, in addition to an increase in flow rate which is regulated through parasympathetic activation, could be a result of the social manipulation factor and so could serve to support Gunther Moor et al.'s propositions further.

The fact that socially rejected participants do not show effects above and beyond socially accepted participants should, however, not be overlooked. It remains possible that this absence of any social rejection specific effects on sAA activity (and reported stress) could be explained further through the methodological pitfalls in the study. While not measuring sAA, Blackhart et al.'s (2007) study collected a saliva sample and measured mood immediately after the social manipulation element, whereas the current design specified that participants wait approximately 8 minutes to complete the 'group' task before the next saliva sample and measurement of stress. As participants had only recently given their baseline measure at the time of the social manipulation, it was considered unwise to repeat the process too quickly for fear that participants would realise the deception. The additional time was also intended to allow participants to ruminate on their respective conditions, with the desired and expected outcome being an amplified feeling of rejection or acceptance (as in Zoccola, Dickerson, & Zaldivar, 2008). However, without a measure immediately following the social manipulation, it is possible that rejected participants specifically did show a transient decrease in sAA activity (to support Gunther Moor et al.'s, 2010, finding), and possibly an increase in reported stress, but that these had recovered within the time gap before the next measurement. This is especially likely considering the fact that participants who might arguably be stronger ruminators of failure, i.e. those who had scored above 50 on the STAI, were excluded from the sample group on ethical grounds. The included sample may, therefore, have been more resilient and so have demonstrated a form of mood repair within the 10-minute interval. Future research should therefore always endeavour to position saliva samples and mood measures as closely to the stressor as possible.

The patterns of response relating to sAA should be interpreted with a degree of caution. The secretion rate of sAA remained unchanged throughout the study, whereas the flow rate of saliva was found to increase. At present, sAA is thought to be independent of

flow rate (Rohleder, Wolf, Maldonado, & Kirschbaum, 2006). However, the argument is far from resolved (see Beltzer et al., 2010) and current advice recommends researchers additionally calculate flow rate when assessing sAA in saliva as a control measure (Salimetrics, 2012). It is possible that the observed changes in sAA activity are a derivative of increased flow rate due, in essence, to the analyte becoming more dilute. For this reason, there can be little confidence in extrapolating such findings beyond the scope of this research study until future research on social stressors has further investigated the source of this pattern of response.

The finding related to cortisol patterns is unexpected as it opposes Blackhart et al.'s (2007) study and the present study's hypothesis. Blackhart et al. found a decrease in cortisol following social acceptance but not social rejection. Blackhart et al. posited that the failure for socially rejected participants' cortisol levels to show the same decrease as participants who were in a control condition or who were socially accepted provided evidence for the stressor being effective by interfering with the natural decline in cortisol levels over time through diurnal variation (e.g. Buchanan, Kern, Allen, Tranel, & Kirschbaum, 2004). Participants who experienced social rejection in this study responded with a decrease in their levels of cortisol, unlike participants who experienced social acceptance whose cortisol levels remained unchanged. Applying the same notion to the current findings would imply that the process of being socially accepted was sufficient to elicit a cortisol response, whilst the decrease in socially rejected participant's cortisol levels was simply a response to natural rhythms. Alternatively, this finding could be interpreted as the stressor being unsuccessful in eliciting a reliable physiological effect, which is supported by the general tendency for laboratory stressors to be largely ineffective in this manner (Dickerson & Kemeny, 2004).

The finding that the condition which was designed to be a comparison group, social inclusion, has in this instance appeared to result in an increase in cortisol is confusing. Aside

from the differences listed above between the present study and Blackhart et al.'s (2007) study, it is unlikely that methodological differences between the two studies are accountable for the discrepancy, as both were conducted over approximately the same length of time in the late afternoon, when cortisol cycles should be less susceptible to circadian fluctuations (Schmidt-Reinwald et al., 1999). In addition to social rejection and social inclusion, Blackhart et al. included a control condition in which participants were informed they had accidentally been assigned to the wrong group and were supposed to complete the "group task" alone. This was different to either of the social manipulation conditions as the reasons behind completing the task alone were inferred as being due to an administrative error rather than positive or negative social evaluation. While Blackhart et al. found no physiological difference between the control and social acceptance condition (hence why here only one was chosen), it is possible that the social inclusion condition failed to act as an appropriate control condition in the present study. However, assuming this to be the case, one would still not expect social inclusion (i.e. positive social evaluation) to lead to increases in cortisol; a stress hormone.

Of critical importance, while not directly related to the study aims or hypothesis, is the finding of a disparity between the first two saliva samples in terms of their overall volume and analyte concentrations. Specifically, flow rate and sAA activity and secretion all increased significantly from baseline 1 to 2, while cortisol concentration was unaffected. This difference is particularly curious in consideration of the fact that just 10 minutes separated the two samples during which participants were chatting as a group, a process initially included to ease participants into the session. While these differences may have occurred due to the effects of interacting within a social environment, it is also possible that they are a result of participants becoming accustomed to the process of donating saliva. This explanation would also account for the lack of any change in cortisol, which is known to be independent of flow

rate. If confirmed, this interpretation might put into question certain conclusions drawn in many studies where, for example, authors attribute (false) changes in biomarkers to psychological interventions. This is especially important given the propensity for the first sample to additionally act as a single baseline. For this reason, before continuing investigating social rejection, cognitive bias and stress further, Study 2 will be dedicated to resolving some of the methodological issues surrounding the collection of saliva for use in biobehavioural research. Specifically, Study 2 will focus on the need for an acclimatisation or 'practice' sample, which will aid future studies contained within this research and the general field by providing evidence as to whether a practice sample should be implemented into research protocols that focus on the acute effects of stress or other manifestations as standard.

To summarise, the present study failed to establish a reliable stressor, that is, one that elicits a resolute psychological and physiological reaction. This aim therefore requires further attention and shall be addressed again in Study 4. There is a potential for the present results to infer partial support for researchers claiming the effects of social stressors (such as social rejection) lead to parasympathetic autonomic command. However, this assumes that decreases in sAA activity were not corrupted here by increases in flow rate. This issue will be further addressed in Studies 2 and 3, which will look at the methodological practicalities of using saliva in research.

4.0 CHAPTER FOUR: STUDY TWO

Investigating the need for a practice sample in salivary biomarker research

The discovery that saliva could act as a biological window, giving snapshot accounts of internal processes in a more convenient and socially permissible way than serum or urine collection, has led to an abundance of studies focusing on how the body physiologically responds to the external environment around them. Early research investigating the physiological effects of stress in the social world has focused mainly on responding levels of cortisol in saliva (e.g. Hellhammer, Heib, Hubert, & Rolf, 1985). As previously discussed, cortisol is released by the adrenal glands following hypothalamic pituitary adrenal (HPA) activation and travels through the blood into saliva via passive diffusion (Vining, McGinley, & Symons, 1983). More recently, sAA has also received considerable interest in stress-related research owing to its close relationship with noradrenaline and consequent potential as a proxy for sympathetic activation (e.g. Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Nater et al., 2005). The finding that salivary sAA increases following periods of acute stress has since been replicated numerous times and appears relatively robust (e.g. Bosch et al. 1996; Chatterton et al., 1996; Nater et al., 2005; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). Prior to this, there was no way of reliably measuring sympathetic activation through salivary biomarkers.

By having biomarkers that represent the two key biological stress responses (HPA and SAM), research is better able to reliably capture the multi-faceted dynamics of the physiological response to stress. However, while convenient, relying solely on one parameter (i.e. saliva) to infer changes to much broader physiological systems increases the risk of misinterpreting the meaning of data. Specifically, changes in analytes might be incorrectly attributed to physiological changes when, in fact, they are brought about due to confounding factors. This study investigates one such potential confound; whether or not practice samples should be implemented into research as standard procedure.

Several factors are known to influence one or both of the key stress-related salivary biomarkers (i.e. cortisol and sAA), and thus need to be considered by researchers when designing experiments and interpreting findings. For example, both biomarkers are governed by natural diurnal rhythms (Kirschbaum & Hellhammer, 1989; Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007), which researchers must be sensitive to in their experimental design. Additional factors include (but are not restricted to) gender (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Kirschbaum, Wust, & Hellhammer, 1992), age (Chahal & Drake, 2007; Strahler, Mueller, Rosenloecher, Kirschbaum, & Rohleder, 2010), exercise exhaustion (Gilman, Thornton, Miller, & Biersner, 1979; Kirschbaum & Hellhammer, 1994), smoking habits (Kirschbaum, Wust, & Strasburger, 1992; Weiner, Levy, Khankin, & Reznick, 2008), and flow rate dependency (Vining, McGinley, & Symons, 1983). Evidence from our laboratory and Study 1 has led to a potential additional confound worth concern; practice effects.

In Study 1 there was a general tendency for participants' initial samples to considerably differ to their subsequent samples, with initial samples tending to be smaller in volume. Assuming both biomarkers are independent of changes in flow rate, meaning that changes in the volume of saliva bear no impact on the concentration of the analyte, this observation should not merit further concern. However, while there is a general understanding that cortisol is independent of changes in flow rate (Kirschbaum & Hellhammer, 1994), there is less consistency in the argument regarding sAA. Supposing that sAA activity is reliant on saliva flow, changes in flow rate could bring about apparent changes in sAA without direct ANS input. In terms of the observation regarding the flow rate of a participant's first sample, such dependence would deem the first measure of sAA as unreliable. This study could therefore provide important evidence for future recommendations of good practice for researchers using salivary biomarkers to measure acute

effects of interventions, such as stress. Further, many studies use the initial sample as a baseline measure against which to compare any post-intervention measures to. If this study is successful in reproducing data to suggest that the first sample might be unreliable, then it is possible that previous interpretations of any changes in biomarkers in response to particular stress/relaxation interventions, specifically those that might be reliant on flow rate, might also be erroneous.

The relationship between sAA and flow rate has been empirically investigated. Early research on unconscious rats discovered that sympathetic activation of the ANS led to a low flow rate of saliva that was richly dense with sAA (Anderson et al., 1984). Alternatively, parasympathetic activation resulted in the opposite pattern; high flow rate containing low amounts of sAA (Asking, 1985). The authors concluded that these findings implied that the release of sAA was influenced by ANS control. However it is possible, instead, that changes in flow rate accounted for or contributed towards the increased or reduced percentage of sAA within a sample. More recently, Rohleder, Wolf, Maldonado, and Kirschbaum (2006) conducted an investigation into the flow/sAA relationship. Male participants underwent a well-known stress test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993) and a control task on consecutive days. Saliva was collected using either the passive drool technique (as was employed in Study 1) or using a Salivette; a cotton swab that absorbs saliva. Rohleder and colleagues noted an increase in flow rate in response to the TSST when saliva was collected using the passive drool method only. In the same group, sAA activity and secretion also increased in response to the TSST, which Rohleder et al. took as evidence that the two measures (flow rate and sAA) were independent. This interpretation was reached because a dependent relationship would result in opposing patterns of response, as was documented following the psychological intervention in Study 1. Consequently, the observed increase/decrease in sAA would have been a result of diluted/stronger concentrations within

the volume of saliva. As both sAA activity *and* saliva flow increased following the social stressor in Rohleder et al.'s study, the two variables do appear in this instance to be independent.

While there has been a general tendency for researchers to take Rohleder et al.'s (2006) findings as evidence that the matter has been resolved (e.g. DeCaro, 2008), others argue that there is insufficient evidence to conclude the debate with any confidence. For example, in their own review on the uses and practicalities of sAA, Rohleder and Nater (2009) continuously refer to flow rate as a potential confound of sAA. Further, current specialist advice (e.g. Salimetrics, 2012) continues to recommend that flow rate should be measured when analysing samples for sAA, thereby suggesting the claim is not entirely assured.

The purpose of the present study is to ascertain whether there is a need to implement a practice sample(s) as standard in research that measures biomarkers that are potentially sensitive to changes in flow rate. Practice samples would be necessary if flow rate is found to be significantly lower in the initial samples, relative to subsequent samples. These aims will be addressed by recruiting participants who have not previously given saliva samples for purposes of research who will receive instructions on how to give a saliva sample. Two groups of participants will then practice the passive drool method of saliva donation (either once or three times), and one group will not practice. Participants will then give four saliva samples using the same method. Salivary biomarkers (flow rate, cortisol concentration, and sAA activity and secretion rate) will be compared over time to determine whether (a) the initial sample from participants who have not practiced the technique is smaller in volume relative to subsequent samples of that group and, if so, whether (b) providing one or three opportunities to practice the technique eradicates this.

It is hypothesised that participants who have no practice sample will show considerable changes in the volume of their four samples; specifically an increase in saliva flow between their first and second samples. Second, despite Rohleder et al.'s (2006) study suggesting otherwise, sAA activity is expected to show a significant decrease between the first two samples in participants who have not practiced the technique. Third, sAA secretion, the calculation of which should correct for changes in saliva flow, is predicted to remain stable during these time points in this sample group. Fourth, based on it being independent of saliva flow, cortisol concentration is predicted to show no change as a result of variation in saliva flow. Finally, participants who have either one or three chances to practice are predicted to show no change in cortisol, saliva flow, or sAA activity or secretion across their four samples.

4.1 Method

4.1.1 Design

This study employed a 3 (condition) x 4 (sampling time points) mixed factorial design (see Figure 5). The independent between-subjects variable was condition assignment: no practice, 1 practice sample, or 3 practice samples. All participants were then required to give four saliva samples (within subjects independent variable), each separated by 10 minutes. Mood was measured at four time points throughout the study and trait measures were taken once to assess potential influences. The dependent variables were flow rate, cortisol concentration, sAA activity and secretion rate, self reported stress, positive and negative affect, and reported optimism, happiness, distress, and tension.

4.1.2 Participants

Staff and students from the University of East Anglia were sent details of the study via email. Those interested in taking part were invited to contact the researcher to receive more detailed information. Sixty-three participants (35 females, 28 males) aged between 19 and 53 years (M = 27.74, SD = 8.88) took part in the study and were randomly assigned to one of three conditions, including a no-practice condition (n =22), one practice sample condition (n =21), and three practice samples condition (n =20). Sessions were run in groups with all participants in one group being in the same condition. Sessions were assigned a condition by alternating between the three conditions (i.e. group 1, group 2, group 3, group 1, group 2, etc). Participants booked into sessions according to their personal availability without prior knowledge of which condition had been assigned to that session. Participants reported having no experience of giving saliva for the purposes of research.

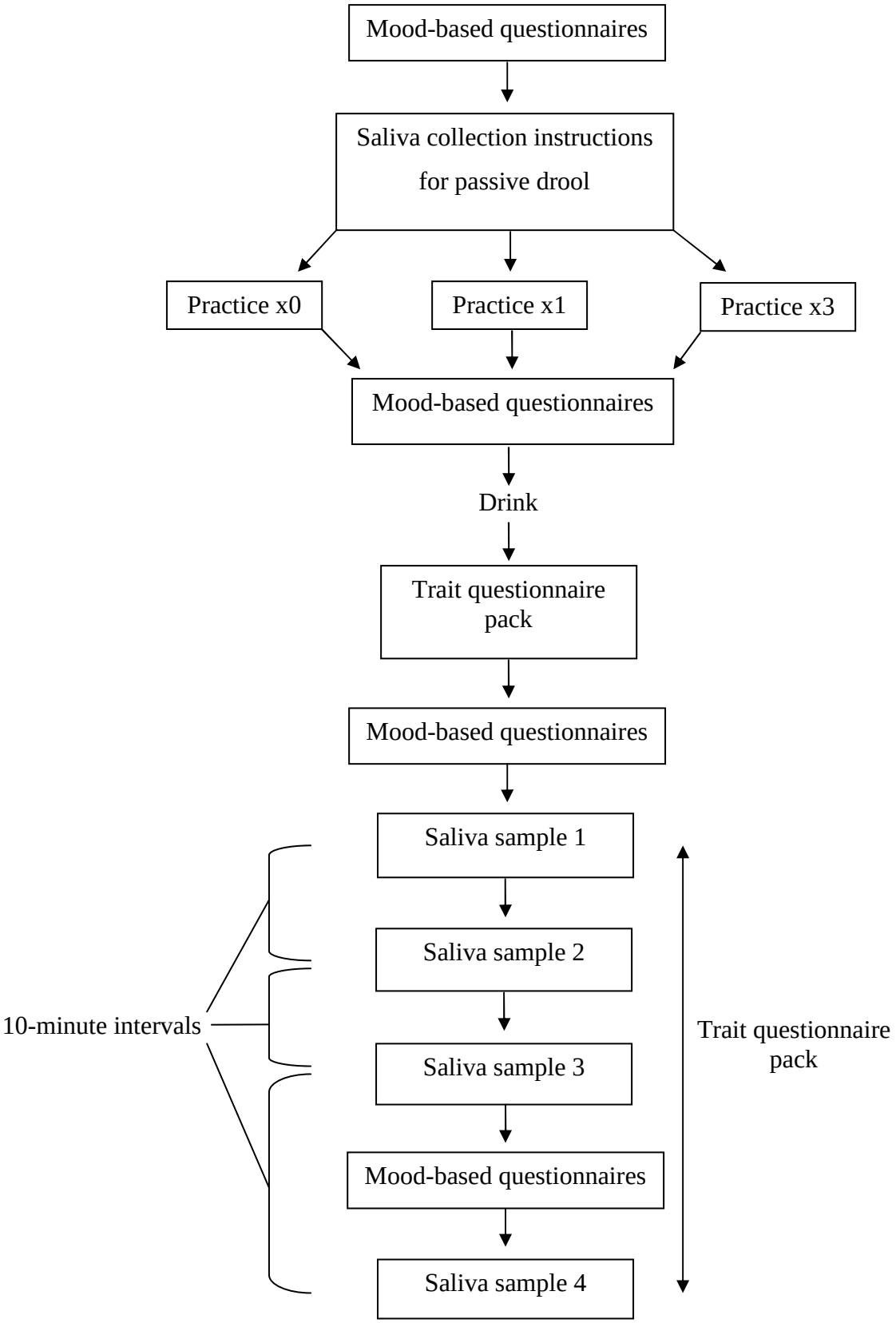

Figure 5. Overview of Study 2's experimental design

4.1.3 Materials

Psychometric measures. ***State assessment.*** Participants completed a series of state-based questionnaires at four time points throughout the study, consisting of the Stress-Arousal Checklist (SACL; Mackay, Cox, Burrows, & Lazzerini, 1978), four visual analogue scales measuring levels of optimism, happiness, distress, and tension (see Study 1 for more details on these scales), and the Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988). The PANAS was designed by Watson et al. to measure two dominant features of emotion; positive affect and negative affect. The scale is composed of 20 adjectives that participants are required to rate on a five-point scale according to their current state. Importantly, these adjectives were chosen due to their independent reference to either positive or negative affect. For example, the term *irritable* describes a degree of negative affect but has a near-zero loading to positive affect. Similarly, *enthusiastic* might describe a state of positive affect but would rarely be used to refer to negative affect. The scale has been shown to have good internal consistency (PA α = .83-.90; NA α = .85-.90) (Watson et al., 1988). The scale is considered to be relatively robust to demographic variables, though Crawford and Henry (2004) noted gender differences. Females reported significantly more negative affect than males, while males reported significantly higher positive affect than females (Crawford & Henry, 2004).

Psychological measures. To fill time between samples and also measure factors that have the potential to influence salivary analytes, participants completed a questionnaire pack consisting of the General Health Questionnaire (GHQ; Goldberg, 1972; Goldberg & Williams, 1988), Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983), Depression Anxiety Stress Scale (DASS; Lovibond & Lovibond, 1995b), a personality inventory (Goldberg et al., 2006), and the Interpersonal Support Evaluation List (ISEL; Cohen & Hoberman, 1983). Study 1 contains further description on each of these scales.

Participants were not required to fully complete this booklet and the data is not referred to in the analysis other than to report participant characteristics where sufficient data exists.

Saliva collection and analysis. Saliva samples were passively collected into 3ml cryovial tubes (Greiner Bio-One Ltd, UK). Samples were frozen at -80°C until required for analysis. Flow rate was determined gravimetrically and samples were assayed for levels of cortisol and sAA (see Chapter two for further details of these procedures).

4.1.4 Procedure

Ethical approval was granted by the Faculty of Health Research Ethics Committee, University of East Anglia. Participants received £8 for their effort and time. All testing sessions were run on weekdays between the hours of 12 – 3pm on campus at the University of East Anglia psychology testing laboratories. Participants were instructed not to eat, drink (other than water) or smoke for 30 minutes prior to the study, and to abstain from undertaking vigorous exercise for 90 minutes prior to participation. On entry to the session, participants were first given information sheets and consent forms before being verbally briefed on the study procedure. Once participants had consented, they completed the first set of questionnaires designed to measure state mood factors. Participants were then given instructions on how to give a saliva sample. The researcher explained the sample tracking procedure, which consisted of writing down unique tube barcode details on a sheet each time a sample was given. Participants were informed that the sample was taken over a 3-minute period, which would be broken down into two 90-second samples. Before each sample, participants were informed they would be asked to clear their mouths by swallowing before sitting with their head tilted forwards to allow any saliva in their mouths to pool at the front. Participants were informed that after a timed 90 second period they would be asked to deposit any saliva through a piece of straw into a cryovial tube. Participants were informed that this

procedure would be repeated a second time into the same tube, through the same straw piece, to complete one sample. Participants were also reassured that they were not expected to fill the tubes and advised not to be concerned about how much saliva they produced compared to other people, as saliva flow rate varies considerably among people. Following the instruction period, two conditions had an opportunity to practice how to give saliva; one condition had one opportunity and one condition had three opportunities. The remaining condition received these instructions but had no opportunity to practice.

Participants then completed the second state assessment before receiving a small drink of still bottled mineral water to clear their mouths of debris and help optimise the quality of the future samples. To prevent the drink influencing the future samples, participants waited 10 minutes before giving another sample. During this time, participants completed the trait questionnaire pack (see *Psychometric Analysis*). Before starting the saliva donation, participants were asked to complete a third state assessment. In all conditions, participants then each gave four samples, which were separated by 10 minutes each during which they returned to completing the trait questionnaire pack. Each sample was taken using the exact procedure that was instructed earlier. Just before the last samples, participants completed a final state assessment. Before leaving, participants were offered some anti-bacterial hand gel and an opportunity to ask any questions or raise any concerns regarding the study.

4.1.5 Data Analysis Plan

Data was explored to check it met the assumptions for parametric testing. Data from trait questionnaires was explored briefly to monitor between-group differences. Data from the state questionnaires was explored to monitor changes in mood throughout the study. To test the study hypothesis, a series of repeated measures ANOVAs were conducted on the relevant

dependant variable data (e.g. reported stress, flow rate, etc), with time as a within subjects factor and group as a between subjects factor. Given gender has been found to also influence salivary biomarkers (e.g. van Stegeren, Wolf, & Kindt, 2008), gender was considered post-hoc to be a potential source of interest and so was included along with condition as a between subjects factor in ANOVAs looking at the effects of having an opportunity to practice giving saliva via the passive drool method. Main effects of time are reported though not necessarily explored where they are qualified by time x group or three-way interactions. For ease of clarity, gender and group main effects, gender x time interactions, and gender by group interactions are largely not reported unless significant or relevant to the point of note. Where appropriate, paired *t*-tests were used to investigate a priori and post-hoc comparisons. Bonferroni corrected alpha levels are reported.

4.2 Results

4.2.1 Data Exploration

The data was explored for outliers and to check it met the parametric assumptions for testing. To successfully normalise the salivary data, which showed a general tendency to be positively skewed and showed platykurtic distribution, all flow rate, cortisol concentration, and sAA activity and secretion data were log transformed. All analyses were conducted using logged data, however descriptive and graphical representation of the means and measures of variation are presented using unlogged data.

4.2.2 Participant Characteristics

A univariate ANOVA revealed no significant difference in age across the three conditions, $F(2, 55) = .95$, $p = .39$, $\eta_p^2 = .03$, or across gender, $F(1, 55) = .86$, $p = .40$, $\eta_p^2 = .02$. Mean levels of self-reported stress (measured by the SACL) on entry to the study were 2.65 ($SD = 3.13$), with no significant difference being found between conditions, $F(2, 57) = .92$, $p = .41$, $\eta_p^2 = .03$, or gender, $F(1, 57) = 1.40$, $p = .24$, $\eta_p^2 = .02$. Chi-squared analyses revealed that the gender ratio was not significantly different across the three conditions, $\chi^2 (2, N = 63) = 1.96$, $p = .38$.

A series of univariate ANOVAs were run on the trait measures taken from questionnaires that revealed no significant main effects of condition or significant condition x gender interactions (see Table 6). A trend main effect of gender was found for the personality subscale agreeableness, $F(1, 56) = 3.60$, $p = .06$, $\eta_p^2 = .06$, with females ($M = 41.06$, $SD = 5.22$) scoring higher than males ($M = 38.29$, $SD = 5.89$). This finding was not considered to be of detrimental effect to future analysis. Instead, the finding further justified the inclusion of gender as a potential confound in analyses.

Table 6

Participant trait characteristics

			Overall (all participants)		Main effect		Gender x condition interaction
					Condition	Gender	
Scale	Factor	*N*	Mean	SD	*p* value	*p* value	*p* value
GHQ	Distress	63	46.33	11.52	.31	.33	.64
PSS-10	Stress	63	21.98	3.39	.74	.16	.79
ISEL	Interpersonal support	63	89.90	16.52	.39	.22	.68
Personality	Extraversion	62	31.71	7.36	.94	.41	.56
	Agreeableness	62	39.81	5.66	.94	**.06**	.30
	Conscientiousness	60	34.68	6.20	.96	.23	.61
	Emotional stability	61	30.93	7.80	.52	.41	.42
	Intellect	63	37.33	5.52	.39	.95	.32
DASS	Depression	63	4.25	3.83	.52	.10	.58
	Anxiety	63	3.94	3.65	.19	.25	.54
	Stress	63	6.97	4.48	.92	.82	.86
	Total	63	30.32	19.55	.47	.74	.72

Note: GHQ = General Health Questionnaire; PSS = Perceived Stress Scale; ISEL = Interpersonal Support Evaluation Checklist; DASS = Depression Anxiety Stress Scale.

4.2.3 Changes in Mood

A 2 (between subjects; gender) x 4 (within subjects; time point) x 3 (between subjects; condition) repeated measures ANOVA was run on the reported state stress (from the SACL). No significant main effect of time was identified, $F(3, 171) = .16$, $p = .93$, $\eta_p^2 < .001$, and there was no significant time x condition interaction, $F(6, 171) = .32$, $p = .93$, $\eta_p^2 = .01$. While there were no significant main effects of condition or gender, and no significant time x gender or gender x condition interactions (all F values < 1), a significant three-way interaction emerged, $F(6, 171) = 2.29$, $p = .04$, $\eta_p^2 = .07$. To explore this, 2 (between subjects; gender) x 4 (within subjects; time) repeated measures ANOVAs were run on data split by condition. For participants who practiced the technique either once or three times, there was no significant main effect of time or significant time x gender interaction (all p values > .15). No significant main effect of time, $F(3, 60) = .39$, $p = .76$, $\eta_p^2 = .02$, or gender, $F(1, 20) = .60$, $p = .45$, $\eta_p^2 = .03$, was found for the no practice condition though a significant time x gender interaction was revealed, $F(3, 60) = 2.85$, $p = .05$, $\eta_p^2 = .13$ (see Figure 6). Efforts were made to investigate this interaction further by running repeated measures ANOVAs on female and male participants within this condition separately, and by running univariate ANOVAs comparing male and female stress scores within this condition at each time point, but no further significant findings emerged (all p values > .11).

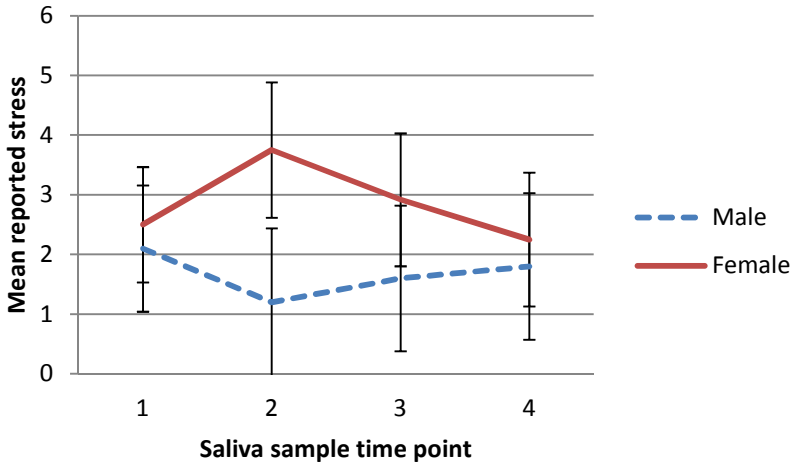

Figure 6. The time x gender interaction for reported stress (SACL) in participants who did not practice giving saliva.

A 2 (gender) x 3 (condition) x 4 (time) repeated measures ANOVA was conducted on reported positive affect, taken from the PANAS. A significant main effect of time was identified, $F(1.83, 93.50) = 11.86$, $p < .001$, $\eta_p^2 = .19$ (Greenhouse Geisser corrected). Post-hoc analysis of the main effect of time on change in positive affect was conducted using paired *t*-tests (see Table 7). A significant decrease in positive affect was identified from time points 1-2, $t(58) = 3.07$, $p = .003$, $d = .20$ (Bonferroni corrected $\alpha = .017$), and 3-4, $t(60) = 2.63$, $p = .011$, $d = .14$, with a trend decrease being revealed from time points 2-3, $t(60) = 2.35$, $p = .022$, $d = .12$. Comparison of the first and fourth time points revealed the largest decrease, from a mean of 27.81 ($SD = 7.40$) to a mean of 23.74 ($SD = 9.51$), suggesting generally that positive affect decreased throughout the study. No significant interactions or main effect of gender was identified (all *p* values > .11). A similar ANOVA was run on the reported negative affect data separately (see Table 7). No significant time, gender, or condition main effects, or significant interactions were revealed (all *p* values > .20).

Table 7

Descriptive data for reported positive and negative affect over time

	N		Time points			
			1	2	3	4
Positive affect	57	Mean	27.81	26.30	25.05	23.74
		SD	*7.40*	*8.42*	*9.26*	*9.51*
Negative affect	57	Mean	12.61	12.05	11.88	11.70
		SD	*3.75*	*3.42*	*3.11*	*2.88*

4.2.4 Hypothesis 1: Saliva Flow

A 2 (gender) x 3 (condition) x 4 (sample time points) repeated measures ANOVA was conducted that revealed a significant main effect of time on saliva flow rate, $F(2.21, 121.68) = 6.65$, $p = .001$, $\eta_p^2 = .11$ (Greenhouse Geisser corrected). No significant condition x time interaction, $F(4.43, 121.68) = 1.24$, $p = .30$, $\eta_p^2 = .04$, but a significant three way interaction between time, condition, and gender was found, $F(4.43, 121.68) = 3.03$, $p = .02$, $\eta_p^2 = .01$.

In order to further explore the significant three way interaction, 3 (condition) x 4 (sample time points) repeated measures ANOVAs were run on male and female participant data separately. For male participants, a significant main effect of time on flow rate was revealed, $F(3, 75) = 3.12$, $p = .03$, $\eta_p^2 = .11$, but no significant main effect of group, $F(2, 25) = .44$, $p = .65$, $\eta_p^2 = .03$, or time x group interaction, $F(6, 75) = 1.22$, $p = .31$, $\eta_p^2 = .01$, was found (see Figure 7a). Paired *t*-tests were carried out but failed to reveal any significant change in flow rate over time (samples 1-2 $p = .08$, samples 2-3 $p = .53$, samples 3-4 $p = .51$; Bonferroni corrected $\alpha = .0167$). For female participants, a significant main effect of time on flow rate was identified, $F(2.03, 61.02) = 4.63$, $p = .01$, $\eta_p^2 = .13$ (Greenhouse Geisser

corrected), that was qualified by a significant time x condition interaction, $F(4.07, 61.02) = 2.90$, $p = .03$, $\eta_p^2 = .16$ (see Figure 7b). To further explore the significant interaction, repeated measures ANOVAs were conducted on female flow rate data from each condition individually[5]. Female participants who had practiced the technique either one, $F(3, 39) = .95$, $p = .42$, $\eta_p^2 = .07$, or three times, $F(1.13, 9.04) = 1.17$, $p = .32$, $\eta_p^2 = .13$ (Greenhouse Geisser corrected), showed no significant change over time in flow rate. Female participants who had not practiced the technique showed a main effect of time, $F(1.26, 11.35) = 4.41$, $p = .05$, $\eta_p^2 = .33$. Paired *t*-tests found a trend increase in flow rate between samples 1-2 in this subgroup, $t(10) = -2.03$, $p = .07$, $d = .66$ (Bonferroni corrected $\alpha = .017$), followed by no change in flow rate between samples 2-3, $t(11) = .64$, $p = .54$, $d = .12$, or 3-4, $t(10) = .63$, $p = .54$, $d = .10$.

To conclude, results appear to partially support the hypothesis regarding the influence of having a practice sample on changes in flow rate. Specifically, females who had no practice sample tended to have more variability in flow rate, with further investigation appearing to support the hypothesis; that an increase in flow rate would be evident between the first two samples for this condition. Female participants who had practiced giving a sample (either once or three times) showed no significant variability in their flow rate.

[5] It is worth noting that as gender did not form part of the primary hypothesis, sample sizes in this further exploration are small: Female no practice group = 10, female 1 practice group = 14, female 3 practice group = 9.

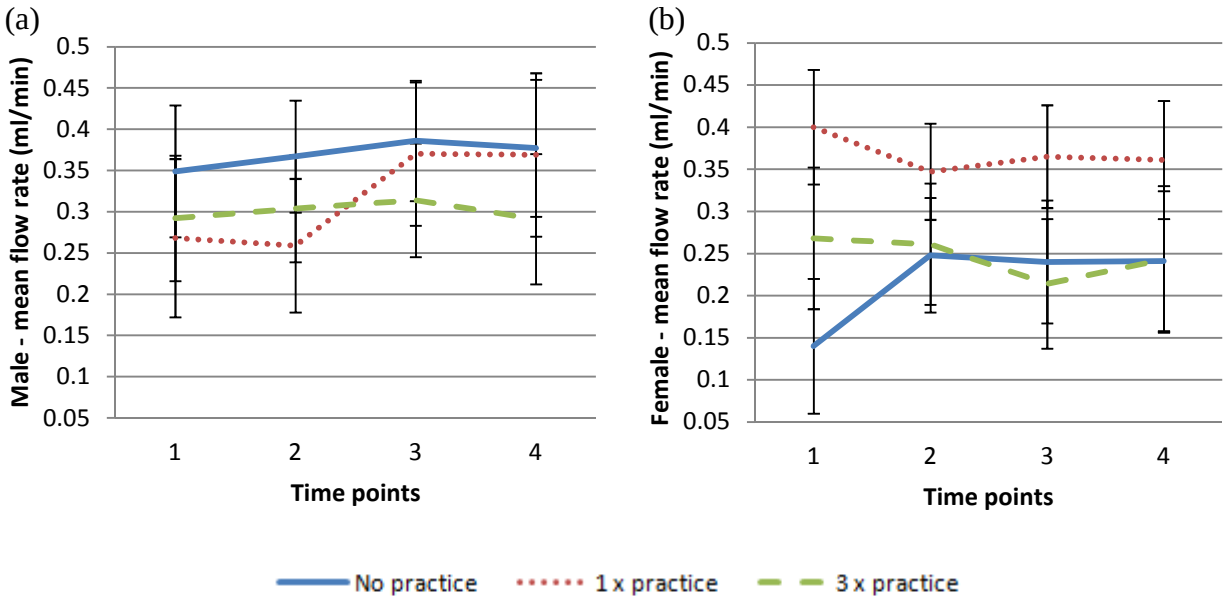

Figure 7. Mean unlogged flow rate changes over time for male participants only split by condition (a) and for females only split by condition (b).

4.2.5 Hypotheses 2 and 3: Alpha Amylase

Hypothesis 2: Activity. A 2 (gender) x 3 (condition) x 4 (sample time points) repeated measures ANOVA showed no significant main effect of time on sAA activity, $F(3, 147) = .67$, $p = .57$, $\eta_p^2 = .01$, and no significant time x group interaction, $F(6, 147) = 1.113$, $p = .35$, $\eta_p^2 = .04$.

Hypothesis 3: Secretion. A 2 (gender) x 3 (condition) x 4 (sample time points) repeated measures ANOVA showed a significant main effect of time on sAA secretion, $F(3, 147) = 3.70$, $p = .02$, $\eta_p^2 = .07$ but no significant time x group interaction, $F(6, 147) = 1.66$, $p = .13$, $\eta_p^2 = .06$. Paired *t*-tests were conducted to further investigate the significant main effect of time (see Table 8) and revealed a trend increase in secretion between samples 1-2, $t(54) = -2.09$, $p = .04$, $d = .18$ (Bonferroni corrected $\alpha = .017$). No significant changes were revealed thereafter (samples 2-3, $t(57) = .15$, $p = .88$, $d = .01$, samples 3-4, $t(57) = -.29$, $p = .77$, $d = .03$).

Table 8

Mean sAA *secretion (U/min) and variation*

	Sample time points			
	1	2	3	4
Mean	9.15	9.89	9.53	10.04
SD	*7.91*	*6.80*	*6.16*	*6.94*

To conclude, sAA activity appeared to remain relatively stable while sAA secretion appeared more variable over time. This did not support the hypothesis, which claimed that sAA activity would mirror changes in flow rate whilst secretion would remain stable.

4.2.6 Hypothesis 4: Cortisol

A 2 (gender) x 3 (condition) x 4 (time point) repeated measures ANOVA was run on participants' cortisol data, revealing a significant main effect of time on change in cortisol concentration, $F(2.32, 113.54) = 11.03$, $p < .001$, $\eta_p^2 = .18$ (Greenhouse Geisser corrected), which was qualified by a significant time x condition interaction, $F(4.63, 113.54) = 2.48$, $p = .04$, $\eta_p^2 = .09$ (see Figure 8). A non-significant trend time x gender interaction was observed, $F(2.32, 113.54) = 2.40$, $p = .09$, $\eta_p^2 = .05$, but there was no significant three way interaction[6], $F(4.63, 113.54) = .92$, $p = .47$, $\eta_p^2 = .04$.

Investigation into the significant time x condition interaction was conducted by selecting data from each condition separately and running repeated measures ANOVAs. For participants who had no practice sample, $F(3, 51) = 7.85$, $p < .001$, $\eta_p^2 = .32$, and one practice sample, $F(1.67, 31.87) = 5.08$, $p = .02$, $\eta_p^2 = .21$ (Greenhouse Geisser corrected), a significant

[6] The trend time x gender interaction was not explored further due to no significant three-way interaction being found. This meant that, while male and female participants' cortisol levels differed at different points across the study, this was not dependent on their condition and thus not of direct interest to the study aims.

main effect of time on cortisol concentration was identified. No significant main effect of time on cortisol concentration was observed in participants who had practiced three times, $F(3, 48) = 1.73$, $p = .17$, $\eta_p^2 = .10$. For the no practice group, paired *t*-tests revealed no change in cortisol between samples 1-2, $t(18) = -.13$, $p = .90$, $d = .02$, or 2-3, $t(18) = -1.27$, $p = .22$, $d = .18$, followed by a trend increase between samples 3-4, $t(18) = -2.13$, $p = .05$, $d = .33$ (Bonferroni corrected $\alpha = .017$). For the one practice group, a trend increase was identified between samples 1-2, $t(19) = -1.88$, $p = .08$, $d = .23$, followed by no change in cortisol between samples 2-3, $t(20) = -1.29$, $p = .21$, $d = .14$, or 3-4, $t(20) = -1.02$, $p = .32$, $d = .09$ (Bonferroni corrected $\alpha = .017$).

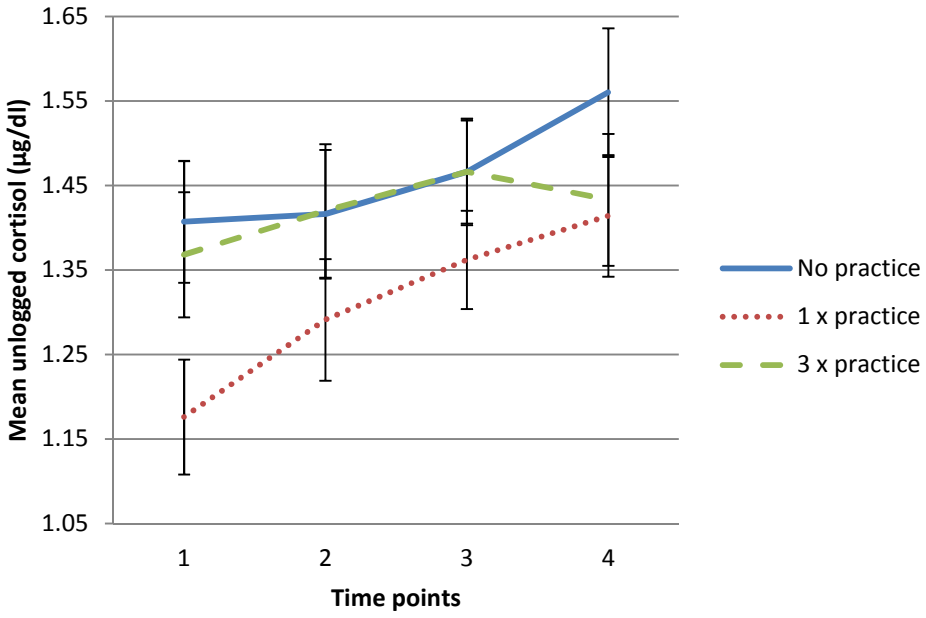

Figure 8. Mean cortisol concentration change across time according to condition

In sum, there was a main effect of time on cortisol concentration which, upon further investigation, appeared to indicate an overall increase in cortisol concentration. This failed to support the hypothesis, which predicted no change in cortisol concentration.

4.3 Discussion

Samples from participants who were not presented with the opportunity to practice giving saliva using the passive drool method were hypothesised to show considerable differences between their first and second (non-practice) samples. Specifically, rate of saliva flow and levels of sAA activity were predicted to significantly increase and decrease respectively. Investigation of a significant three-way (time x condition x gender) interaction provided tentative support for the hypothesis in flow rate. Evidence of patterns in the predicted direction was found in female (but not male) participants who did not practice the technique. There was no evidence of change in sAA activity over time, or any time x condition interaction or three-way interaction, which failed to support the hypothesis in light of the findings in flow rate. There was a main effect of time of sAA secretion though no time x group interaction. This supports the hypothesis, which predicted no condition-dependent variation. Contrary to the hypothesis, a time x condition interaction was found for cortisol concentration. Cortisol appeared to generally increase across the study, though this was found not to be significant in participants who had practiced giving saliva three times.

While the increase in cortisol concentration across the study appeared to be limited to participants who had not practiced giving saliva or who had practiced once, further consultation of the means might offer a potential reason for this. When inspecting Figure 8, mean cortisol concentration appears to increase in all conditions from samples 1-3, though there appears to be a decline in concentration between samples 3-4 for participants who have had three practice samples. In hindsight, this fourth sample could be considered as superfluous, as the hypothesis related more to the first and second samples. Samples 3 and 4 were included to monitor the after effects of any significant changes found between samples 1 and 2. As cortisol concentration appears to increase between samples 1-3 in the three practice condition, it is proposed that the significant time x condition interaction that is

documented in the results section is more the result of an anomaly rather than evidence of practice effects in cortisol.

The finding that females specifically seem to be more sensitive to changes in flow rate when they are not afforded any chance to practice the passive drool technique could suggest that the effects are due to participants needing time to become more comfortable with the procedure rather than them learning to use the method correctly with practice. This notion seems likely in consideration of the fact that females have been shown to exhibit a greater physiological stress response to stressors that include an element of social threat compared to males (e.g. Salvador, 2005; Stroud, Salovey, & Epel, 2002). This is thought to be owing to their comparatively greater evolutionary social role (Taylor et al., 2000) and the tendency for women to define themselves through their social relationships relative to men who focus more on their independent achievements (Cross & Madson, 1997). Saliva flow is currently understood to be regulated through the ANS (Garrett, 1987), with parasympathetic dominance leading to increases in flow rate and sympathetic dominance linked with decreases in flow rate. Consequently, it could be argued that female participants found the experience of giving saliva samples in a public setting more embarrassing or felt a minor degree of stress relative to male participants. This could have led to the patterns evidenced in the present study following temporary sympathetic command.

In a bid to either confirm or dispute the above argument, it would seem wise to refer to the patterns of response in reported stress or positive or negative affect across the study generally. However, these measurements were not designed to assess the reaction to specific events in the current study, such as the process of giving a first sample for females who had no prior experience, and as such were not ideally placed to capture such a response. For example, while the third mood measurement might provide an accurate baseline for this sub-sample by being positioned just before the first saliva sample, the fourth measurement of

mood is not taken until just before the fourth and final saliva sample, by which time any acute effects would arguably have diminished. Therefore it seems that the lack of change in the self report data might not be reason to discard the interpretation. However, supposing that females who had not practiced the method did show variability in their flow rate owing to getting used to the social situation, one might reasonably expect some fluctuations in sAA during this period due to its current standing as a marker for sympathetic activity. Conversely, the results document no significant change in sAA activity or secretion between samples 1-2 in this sub-sample, which could provide a counter argument to this theory. Future research might seek to address this issue through improved positioning of the mood measures to capture any subtle or significant changes in state reflection.

The absence of any condition-dependent changes in sAA secretion when condition-dependent changes in flow rate were present supported the hypothesis. This is explained by the fact that secretion rate (sometimes referred to as output) accounts for changes in flow rate in its calculation and so is often considered in addition to measures of sAA activity. However, the absence of a change in sAA activity as a direct result of changes in flow rate completely opposes the experimental hypothesis, which was based on findings from Study 1. Indeed, the findings of the present study support work by Rohleder, Wolf, Maldonado, and Kirschbaum (2006), who posit that sAA activity is independent of flow rate. It should be noted, though, that the flow rate patterns of response in the present study were found in a sample consisting of just 10 participants, which significantly reduces the statistical power of the analysis. Further research using larger sample sizes of females and males is recommended before drawing any firm conclusions regarding the relationship between flow rate and sAA. For the purpose of future research within the span of this thesis, the inconclusive findings should lead future studies including saliva samples to err on the side of caution and include a

sample dedicated purely to the purpose of practicing the method of saliva donation, unless published research or specialist advice recommends otherwise.

In conclusion, the present study appeared to unearth some interesting findings suggesting that research using female participants might benefit from introducing a practice sample as standard to provide reliable flow rate data. Contrary to expectations and the findings of Study 1, sAA activity was not found to oppositely mirror changes in flow rate, which could be taken to suggest either that the two are independent of each other or that the flow rate data is indeed fallible due to its small sample size. As predicted, the opportunity to practice did not influence variability in either cortisol or sAA secretion. This finding suggests that the implementation of a practice sample as standard would have no bearing on these two measures specifically. Taken together, these findings do not completely clarify the utility of a practice sample, with some data demonstrating its necessity and some data suggesting it to be an irrelevant factor. It therefore seems prudent that future studies contained within this thesis should endeavour to incorporate a practice sample, as standard procedure for accurate and reliable saliva collection. However, for application outside of the scope of this thesis, more research is needed to further address this issue in light of the small sub-sample within which this pattern emerged.

5.0 CHAPTER FIVE: STUDY THREE

Saliva collection techniques: Comparing passive drool with collection via an oral swab

The findings of Study 1 highlighted some practical concerns relating to obtaining accurate and reliable measures of saliva for the assessment of biological markers of acute stress. Specifically, the first two samples that participants donated showed great variation in terms of sample volume and analyte levels. Study 2 explored these issues by investigating the necessity of providing participants with an opportunity to practice the collection method (passive drool) prior to collecting a baseline sample. Results from Study 2 suggested a gender bias, with samples from female participants showing evidence intimating that a practice sample would be beneficial. Alternatively, this pattern was absent in samples taken from male participants. Prior to continuing with further work aimed at establishing the link between cognitive biases and the stress response, Study 3 pursued an alternate line of interest with the aim of producing a standard procedure for saliva collection for the remaining studies within this thesis. Specifically, the present study compared two common methods of collecting saliva; passive drool (as employed in Studies 1 and 2) and using a commercially available absorbent insert, a Salimetrics Oral Swab (SOS; Salimetrics LLC, USA).

The SOS is a relatively new device for researchers, with manufacturers claiming it to be an interference-free (for the majority of analytes) inert insert that produces a clear and workable sample (Salimetrics, 2011). Whereas the passive drool method of saliva collection requires participants to donate “whole” saliva (i.e. not gland specific) directly into a tube, the SOS insert is placed in the mouth and absorbs saliva present. For this reason, saliva collection using an SOS enables researchers to target specific glands known to release dense amounts of certain analytes. For example, placement of the swab adjacent to the parotid gland would be advisable for targeting sAA.

The SOS additionally works as a filter, as the swab retains the majority of the sample debris meaning that sample expressed from the swab during centrifugation is clear and workable. This permits smaller volumes to be utilised with a greater degree of accuracy,

resulting in fewer samples having to be discarded owing to low volume. In a practical sense, this ability to artificially filter the saliva has the potential to dramatically improve the utility of a sample when compared to saliva collected using the passive drool technique, which is commonly cloudy or contains obvious debris, such as phlegm, prior to centrifugation. For saliva collected through passive drool, the process of centrifugation pellets sample debris at the bottom of the tube leaving a clear workable sample at the top. This procedure successfully produces a readily utilisable sample providing the original sample volume is sufficiently large. Difficulties can be encountered when samples contain only a small volume. This is because there is a smaller range for error between the clean and "dirty" divisions of the sample, which increases the chance of encountering pipetting errors such as aspirating some of the sample debris. Due to such reliability issues, passive drool samples with particularly small volumes are often discarded.

The arguments above imply that collecting saliva using a SOS provides a sample of superior functional quality relative to using the passive drool method. Further, saliva donation using an absorbent swab has been shown to be preferable from a participant's perspective over being told to 'drool' or 'spit' into a tube (Strazdins et al., 2005). However, it is far costlier to use and some researchers maintain cautious reservations about its use owing to past errors. For example, a similar aid to the SOS is a cotton swab commonly referred to as a Salivette (Sarstedt, Germany). The Salivette has been used by many researchers to collect saliva to quantify levels of cortisol amongst other biomarkers. Only relatively recently, research has provided evidence suggesting that Salivettes directly interfere with the sample to produce a biased result (e.g. Bristow, Cook, Edwards, & Veerapen, in prep). Essentially, the post-centrifugation workable sample has been found to be slightly altered compared to the original sample that was collected from the participant's mouth, insinuating that the swab somehow interferes with certain analytes. There is evidence to suggest that this suspicion

emerged some time ago. For example, Aufricht et al. (1992) observed a reduction in the recovery of a salivary immune marker, Immunoglobulin-A (IgA), following the use of Salivettes relative to using the spitting method (which is a form of passive drool in which participants are required to rapidly expectorate, or spit, samples into a tube). Regarding other analytes, it appears this bias was either not necessarily present or, more ominously, less predictable. For example, Shirtcliff, Granger, Schwartz, and Curran (2001) collected saliva samples from participants using the passive drool method. The sample was then divided, with half passed through a Salivette and half left as a control sample. While the results showed evidence of a Salivette-induced interference in a number of analytes, including testosterone, IgA, and progesterone, they concluded that cortisol was unaffected by the collection method. However, Strazdins et al. opposed this conclusion by finding significantly reduced levels of cortisol concentration following the use of Salivettes as a collection device compared to passive drool. Again focusing on the measurement of IgA, Bristow et al. present four experiments demonstrating a severe and unsystematic bias caused by Salivettes which further posit that the bias seems to be proportional to the volume of the sample, with smaller volumes leading to greater errors.

An additional key concern regarding the use of SOS to collect saliva relates to whether or not the insert stimulates saliva flow. Standard guidelines for the previously used Salivettes recommended that participants chew on the swab during saliva collection. This process would artificially stimulate saliva flow by mimicking gustatory movements of the jaw (Humphrey & Williamson, 2001), which would typically result in an increase in saliva flow from the glands that are most involved in the digestive process; the parotid glands in the cheek. Indeed, Humphrey and Williamson found that the relative contribution from the parotid gland to overall saliva composition increased from approximately 20% under resting or unstimulated conditions to over 50% following stimulation in this manner. As previously

discussed (see Study 2), the debate concerning sAA's independence to saliva flow has yet to be concluded, though Rohleder, Wolf, Maldonado, and Kirschbaum (2006) claim that sAA is independent of flow rate. However, as it is primarily an enzyme involved in the breakdown of food (Schenkels, Veerman, & Nieuw Amerongen, 1995), sAA is synthesised in the acinar cells of the parotid gland. Therefore, specific activation of this gland (e.g. through chewing a swab) would arguably lead to a large increase in the secretion of sAA. For this reason, Bosch, Veerman, de Geus, and Proctor (2011), oral biologists, suggest that the collection saliva with the aid of swabs for the measurement of such analytes is inadvisable due to the sample being unrepresentative of the whole picture and, hence, possibly invalid. However, Salimetrics (2011) disagree and claim that reliable levels of sAA can be collected (in addition to other analytes) using the SOS providing the swab is placed and held under the tongue. This would target absorption of saliva secreted more from the sublingual major salivary gland, which would arguably be less subject to this digestive bias. Additionally, by keeping the jaw still during the collection, Salimetrics claim that the parotid glands should not be artificially activated therefore avoiding the bias that Bosch et al. suggest.

The present study aims to devise a standard method of saliva collection for use in the remaining studies within this thesis. To achieve this, samples collected using the passive drool technique will be compared with samples collected with the aid of the SOS in terms of flow rate, cortisol concentration, and sAA activity and secretion. In consideration of the findings from Study 2 regarding the need for a practice sample, participants will practice both methods before giving one respective sample per method. When giving a saliva sample using the SOS, participants will place the swab under their tongue and keep it there for two minutes, which is the advised time to prevent the swab from becoming entirely saturated with saliva as this would make any saliva flow calculation (and, subsequently, sAA secretion calculation) inaccurate. As the present study aims to sample the same type of saliva (i.e.

unstimulated), it is hypothesised that (1) there will be no difference (and no influence by gender) between the two methods of saliva collection on saliva flow, cortisol concentration, or sAA activity or secretion. Further, and for the same reasons, it is hypothesised that (2) significant positive correlations will emerge between the two methods for each of the physiological dependent variables listed above.

5.1 Method

5.1.1 Design

The study was a 2 x 2 x 2 mixed factorial design, with saliva collection method (SOS or polypropylene cryovial) as a within participants factor and order of method (i.e. which technique was used first) and gender (male or female) as between participants factors (see Figure 9). Participants were given a practice sample for each method and were required to give one sample using each method. Mood was measured at three time points throughout the study and psychological trait measures were assessed once to monitor potential influences and confounds. The dependent variables from the scales measuring state well-being were self reported stress, positive and negative affect, optimism, happiness, distress, and tension. From the saliva samples, the dependent variables were flow rate, cortisol concentration, and sAA activity and secretion rate.

5.1.2 Participants

Sixty four volunteers (35 females, 24 males and 5 unspecified) aged 18-59 years were recruited from an undergraduate and staff population at Anglia Ruskin University, Cambridge, via an email advertisement and posters displayed across the campus. The order by which participants used each method to give a sample was determined by which session they took part in. Participants booked into sessions based on their availability, with each session alternating which technique was used first.

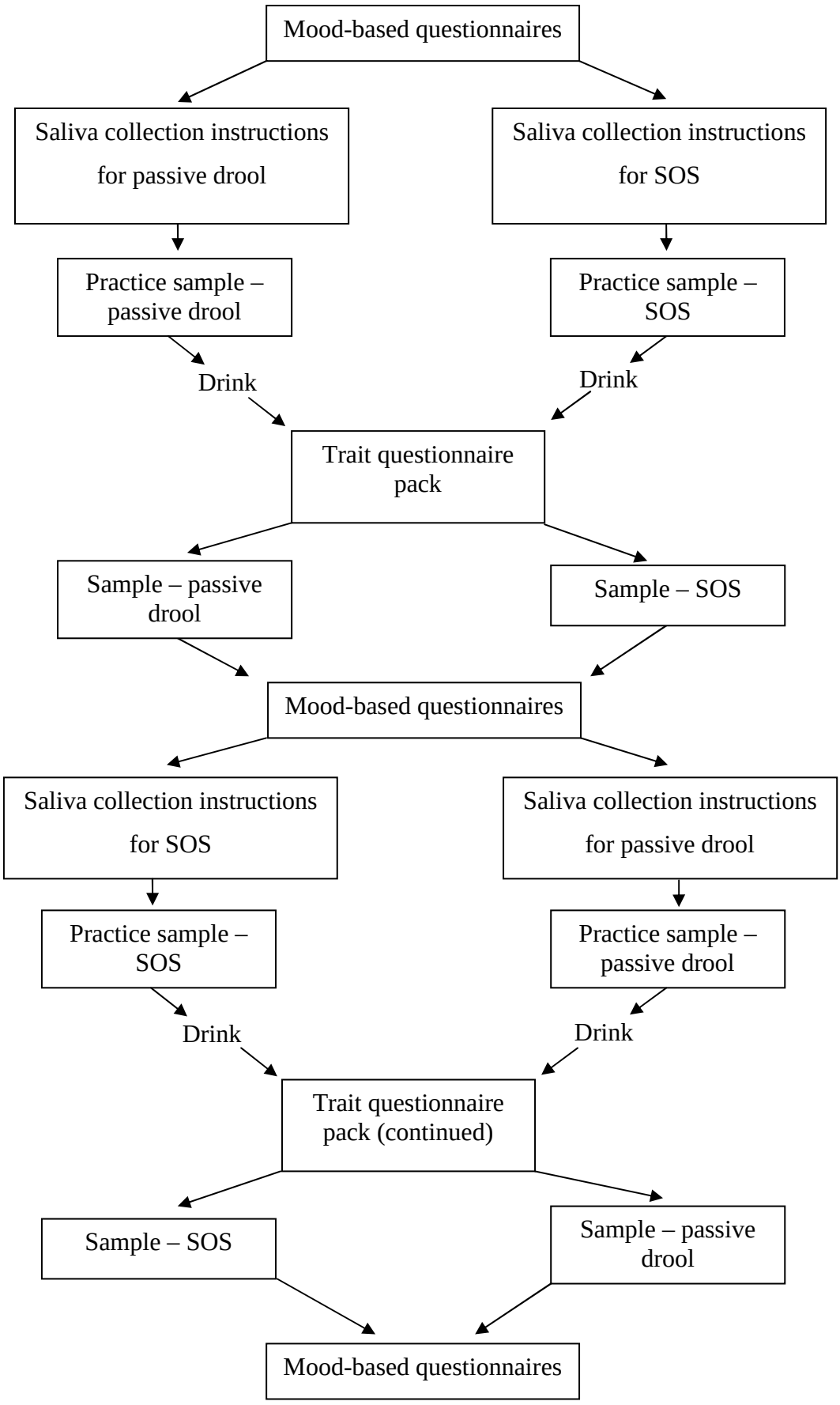

Figure 9. Overview of Study 3's experimental design

5.1.3 Materials

Saliva collection. Saliva was collected using an absorbent SOS or into a cryovial via passive drool. Details on saliva analysis procedure can be found in Chapter two.

SOS collection. Each participant had one opportunity to practice and then gave one sample using a SOS (Salimetrics LLC, US). The SOS is made from an inert food grade material, and so is safe if oral consumption were to occur. Participants were told to clear their mouths by swallowing before placing the swab under the tongue for a period of precisely two minutes (timed). Following this, swabs were placed into a storage tube and stored at -80ºC until analysis.

Cryovial collection. Each participant had one opportunity to practice and then gave one sample using a passive drool technique into a cryovial tube (Greiner Bio-One Ltd, UK). Participants were told to clear their mouths by swallowing before sitting with their head tilted forwards to allow any saliva in the mouth to pool at the front for a period of precisely 90 seconds (timed). Following this, participants were instructed to deposit pooled saliva into a cryovial tube with the aid of a piece of straw. This process was repeated using the same tube and straw piece to produce one sample. The piece of straw was then discarded while the cryovial was stored at -80ºC until analysis.

Psychological measures. ***Mood measures.*** Participants completed a series of state questionnaires three times throughout the study (see Studies 1 and 2 for more details on each of these scales): upon entry (baseline), and following each of the two (non-practice) saliva samples. The questionnaires included the Stress Arousal Checklist (SACL; Mackay, Cox, Burrows, & Lazzerini, 1978), Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988), and four VAS with terminals labelled *depressed* to *happy*, *pessimistic* to *optimistic*, *distressed* to *not distressed*, and *tense* to *relaxed.*

Character measures. Participants additionally completed a questionnaire pack containing questions related to health behaviour (e.g. amount of alcohol consumed over the previous week), compliance with instructions given relating to the hours leading up to the study session (e.g. time last ate/drank/smoke), the General Health Questionnaire (GHQ-28; Goldberg, 1972; Goldberg & Williams, 1988), Perceived Stress Scale (PSS-10; Cohen, Kamarck, & Mermelstein, 1983), Interpersonal Support Evaluation List (ISEL; Cohen & Hoberman, 1983), a personality inventory (Goldberg et al., 2006), and the Depression Anxiety Stress Scale (DASS; Lovibond & Lovibond, 1995b). Details of all of these questionnaires are outlined in Study 1.

5.1.4 Procedure

The study was approved by Anglia Ruskin University's Research Ethics Subcommittee. Participants were asked to refrain from eating, drinking (other than water), and smoking for 30 minutes prior to the study, and to abstain from undertaking vigorous exercise for 90 minutes prior to the start. After giving written informed consent, participants completed a set of the mood measures. Participants were then given instructions detailing one method of giving a saliva sample (either using a SOS swab or via passive drooling into a cryovial). Participants practiced the procedure for one of the techniques before receiving a drink of mineral water to clear their mouth of any debris. Participants then waited 10 minutes to enable the salivary glands to normalise during which they started to complete the character questionnaire pack. After 10 minutes had elapsed, participants gave one saliva sample using the method previously practiced. Following this, participants completed a second set of mood measures. Participants then repeated the above procedure using the remaining method of saliva sampling (either using a SOS swab or via passive drooling into a cryovial). Specifically, participants received instructions on the technique before having a practice sample. They then were issued with a drink of bottled mineral water, before waiting 10

minutes (working again on the questionnaire pack). Participants then gave a second saliva sample, using the second technique. Participants completed a third and final set of mood measures before being thanked and paid £5 for their effort and time.

5.1.5 Data Analysis Plan

Data was explored prior to analyses to ensure it met the assumptions of parametric testing. Participant characteristics from the trait questionnaires were compared between groups to determine whether successful randomisation had occurred. Data from the state questionnaires was investigated to monitor changes in mood over the study. Repeated measures ANOVAs with time as a within-subjects factor were used to test the first hypothesis. In light of the findings from Study 2 and others suggesting gender to be a potentially confounding variable in salivary biomarker analyses (e.g. van Stegeren, Wolf, & Kindt, 2008) gender was entered along with condition (the order of collection technique) as between-subjects variables. Main effects of technique are reported and explored. For ease of clarity, gender and condition (order) main effects, technique x condition, technique x gender, and condition x gender interactions, and three-way (technique x condition x gender) interactions are largely not reported unless significant or relevant to the point of note. Where appropriate, paired *t*-tests were conducted to investigate a priori or post-hoc rationalisations. Hypothesis two was investigated using bivariate correlations to investigate the relationships between the two methods.

5.2 Results

5.2.1 Data Exploration

Three participants' data were removed and excluded from all analysis on the basis of being largely incomplete. All variables were explored to check they conformed to the assumptions of parametric testing. All physiological variables (flow rate, cortisol concentration, and sAA activity and secretion) were log transformed to achieve normal distribution. All analyses were conducted using logged data, however descriptive and graphical representation of the means and measures of variation are presented using unlogged data.

5.2.2 Participant Characteristics

There was no significant difference between the number of males and females assigned to each order condition (which technique they used first), χ^2 (1, N = 59) = .73, p = .39. Univariate ANOVAs were carried out on participants characteristic data (see Table 9). A main effect of gender was found for the DASS stress subscale, F(1, 38) = 4.70, p = .04, η_p^2 = .11, and the personality variables extroversion, F(1, 42) = 5.63, p = .02, η_p^2 = .12, and conscientiousness, F(1, 42) = 9.01, p < .01, η_p^2 = .18. Females scored significantly higher on the conscientiousness (M = 37.00, SD = 6.40) and DASS stress (M = 14.76, SD = 8.94) scales relative to males (conscientiousness M = 31.18, SD = 5.07; DASS stress M = 9.54, SD = 6.98). Alternatively, males were significantly more extrovert (M = 37.71, SD = 8.42) than females (M = 30.97, SD = 7.00). A significant main effect of order of technique (condition) was found for the personality subscales extroversion, F(1, 42) = 4.40, p = .04, η_p^2 = .10, and intellect, F(1, 42) = 4.05, p = .05, η_p^2 = .09. As a group, participants who used the cryovial method to give saliva first scored significantly higher on both scales (extroversion M = 35.75,

SD = 9.40; intellect *M* = 44.08, *SD* = 6.33) relative to those who used the SOS method first (extroversion *M* = 30.95, *SD* = 5.76; intellect *M* = 40.68, *SD* = 6.20).

Table 9

Descriptive data of participant trait characteristics

Scale	Characteristic	*N*	Overall (all participants)		Main effect		
					Order of technique (condition)	Gender	Condition x Gender interaction
			Mean	SD	*p* value	*p* value	*p* value
GHQ-28	Distress	59	20.15	10.36	.62	.39	.67
PSS-10	Stress	53	24.47	6.70	.19	.45	.70
ISEL	Interpersonal support	56	82.63	19.73	.61	.42	.52
DASS	Depression	42	7.67	8.78	.14	.40	.25
	Anxiety	42	6.95	5.75	.95	.81	.98
	Stress	42	13.14	8.65	.13	.04	.66
Personality	Extroversion	46	33.46	8.16	.04	.02	.11
	Agreeableness	47	39.21	4.91	.79	.31	.11
	Conscientiousness	46	34.85	6.53	.79	.005	.99
	Emotional stability	44	28.30	6.44	.95	.41	.17
	Intellect	46	42.46	6.43	.05	.12	.15

Note: GHQ = General Health Questionnaire; PSS = Perceived Stress Scale; ISEL = Interpersonal Support Evaluation Checklist; DASS = Depression Anxiety Stress Scale.

5.2.3 Changes in Mood

A 3 (time point) x 2 (order) x 2 (gender) repeated measures ANOVA was run individually on reported stress (SACL), positive affect, negative affect (PANAS), and the four visual analogue scale measures (optimism, happiness, distress, and tension). A significant main effect of time was identified for stress, $F(1.40, 77.15) = 5.45$, $p = .01$, $\eta_p^2 = .09$ (Greenhouse-Geisser corrected), optimism, $F(2, 102) = 4.14$, $p = .02$, $\eta_p^2 = .08$, and happiness, $F(1.58, 80.41) = 5.48$, $p = .01$, $\eta_p^2 = .10$. Further investigations of these main effects revealed a significant decrease in reported stress between the first two measures, $t(60) = 3.07$, $p = .003$, $d = .27$ (Bonferroni corrected $\alpha = .0167$) from an average reporting of 5.03 ($SD = 4.75$) to 3.70 ($SD = 4.94$). There was no significant change in reported stress between the final two measures, $t(60) = .13$, $p = .90$, $d = .01$. For optimism, there was no significant change between measures 1 – 2, $t(57) = -.47$, $p = .64$, $d = .04$, or 2 – 3, $t(56) = -2.25$, $p = .03$, $d = .15$, but revealed an overall significant increase in reported optimism between measures 1 – 3, $t(56) = -2.71$, $p < .01$, $d = .21$, from an average reporting of 6.94 ($SD = 2.20$) to 7.41 ($SD = 2.31$). Similarly, for happiness ratings, there was no change between the measures 1 – 2, $t(57) = -2.11$, $p = .04$, $d = .13$, or 2 – 3, $t(56) = -1.84$, $p = .07$, $d = .12$, but an overall significant increase over the study period as ascertained by the significant increase from measure 1 – 3, $t(56) = -2.98$, $p < .01$, $d = .27$, from an average reporting of 6.89 ($SD = 2.40$) to 7.51 ($SD = 2.18$).

While no significant main effects of time emerged for positive or negative affect (both F values < 1), a significant main effect of gender was found for positive affect, $F(1, 46) = 4.59$, $p < .05$, $\eta_p^2 = .09$, and a trend effect found for negative affect, $F(1, 44) = 3.39$, $p = .07$, $\eta_p^2 = .07$. Males participants reported higher positive affect ($M = 31.62$, $SD = 8.18$) and negative affect ($M = 15.30$, $SD = 5.95$) relative to female participants (positive affect $M =$

26.25, *SD* = 9.71; negative affect *M* = 12.85, *SD* = 3.98). No significant main effects or interactions were present in the VAS distress or tension scales.

5.2.4 Hypothesis One

Flow rate. A 2 (collection method) x 2 (order) x 2 (gender) repeated measures ANOVA revealed no significant main effect of collection method, $F(1, 55) = .07$, $p = .79$, $\eta_p^2 = .001$. A significant collection method x gender interaction was identified, $F(1, 55) = 4.46$, $p = .04$, $\eta_p^2 = .08$ (see Figure 10). To investigate this interaction, repeated measures ANOVAs were run on male and female data separately, using collection method as a within subjects factor. No significant main effect of collection technique was found for female participants, $F(1, 34) = 1.98$, $p = .17$, $\eta_p^2 = .06$, or male participants, $F(1, 23) = 2.31$, $p = .14$, $\eta_p^2 = .09$. Univariate ANOVAs were also conducted on flow rate data from SOS and cryovial methods separately with gender as a between subjects factor. No significant main effect of gender was found for flow rate collected either through SOS, $F(1, 57) = 1.32$, $p = .26$, $\eta_p^2 = .02$, or cryovial, $F(1, 57) = 1.51$, $p = .22$, $\eta_p^2 = .03$, techniques.

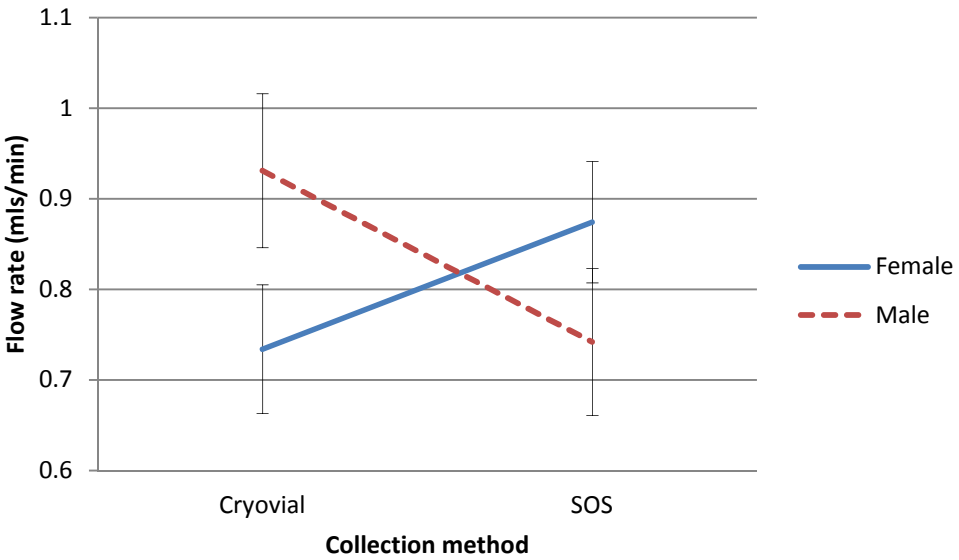

Figure 10. The significant collection method x gender interaction for flow rate.

Cortisol. A 2 (collection method) x 2 (order) x 2 (gender) repeated measures ANOVA revealed a significant main effect of collection method on cortisol, $F(1, 53) = 8.71$, $p = .005$, $\eta_p^2 = .14$. Cortisol concentration was found to be significantly higher when saliva was collected via SOS insert ($M = .24$µg/dl, $SD = .42$) relative to when collected into a cryovial ($M = .16$µg/dl, $SD = .11$). A significant main effect of gender was also identified, $F(1, 53) = 12.49$, $p = .001$, $\eta_p^2 = .19$, with males producing more cortisol ($M = .29$µg/dl, $SD = .37$) relative to females ($M = .13$µg/dl, $SD = .08$).

Alpha amylase. A 2 (collection method) x 2 (order) x 2 (gender) repeated measures ANOVA was conducted on sAA activity and secretion data separately. No significant main effects of technique or order, technique x order, technique x gender, order x gender interactions, or three-way interactions were found (all p values > .12). No main effect of gender was found for sAA activity, $F(1, 47) = .94$, $p = .34$, $\eta_p^2 = .02$, though a trend main effect of gender was found for sAA secretion rate, $F(1, 47) = 3.04$, $p = .09$, $\eta_p^2 = .06$, with males showing a slightly higher rate of secretion relative to females.

5.2.5 Hypothesis Two

Flow rate. A Pearson's correlation revealed a significant positive relationship between flow rate when measured by passive drool and flow rate when measured by SOS, $r(61) = .29$, $p = .03$.

Cortisol. A significant positive relationship was found between cortisol concentration measured through passive drool and cortisol concentration measured through SOS, $r(59) = .78$, $p < .001$.

Alpha amylase. Person's correlation identified significant positive relationships between the two collection methods in terms of both concentration, $r(53) = .83$, $p < .001$, and

secretion, $r(53) = .70$, $p < .001$. Further, a significant positive relationship was observed between concentration and secretion data for both cryovial, $r(55) = .89$, $p < .001$, and SOS, $r(53) = .90$, $p < .001$, methods.

General. While significant positive relationships were identified between the two collection techniques for the measures noted above, the correlation coefficients differed significantly between flow rate and the salivary biomarkers (see Table 10).

Table 10

Correlation coefficients for measures assayed through samples collected via passive drool or SOS techniques

		sAA	
	Cortisol	Concentration	Secretion
Flow rate	**$z = 4.04$, $p = .001$**	**$z = 4.57$, $p < .001$**	**$z = 2.93$, $p < .01$**
Cortisol		$z = -.64$, $p = .52$	$z = .98$, $p = .33$
sAA activity			$z = 1.58$, $p = .11$

Summary

Participants reported no change in their positive or negative affect, distress, or tension across the study, whilst a decrease in state stress was identified from the beginning to the middle of the study alongside a general increase in happiness and optimism. The technique used to collect saliva did not appear to significantly affect measurements of sAA activity or secretion, whilst higher levels of cortisol concentration were found when participants collected saliva using the SOS insert relative to using a cryovial. Males were found to report

significantly higher levels of affect (both positive and negative) and were shown to release significantly higher amounts of cortisol and secrete slightly (trend) more sAA. Significant positive correlations were identified for all of the salivary biomarkers when investigating the relationships between the two collection methods, though *z* scores indicated that the correlations for flow rate were significantly different from correlations for cortisol or sAA (activity or secretion).

5.3 Discussion

The finding of no significant main effect of collection method in addition to significantly positive correlations between the two techniques for both sAA activity and secretion support the study's two hypotheses. Flow rate also showed no significant main effect of collection method. However, a significant gender x collection method interaction emerged for flow rate. Further investigation failed to clarify this interaction. Regardless of this, a significant positive relationship was identified for flow rate when examining the relationship between the two methods, thus supporting hypothesis two. In contrast to hypothesis one, a significant main effect of technique revealed a significantly higher level of cortisol concentration in samples collected using SOS relative to passive drool samples. However, a significant positive relationship still emerged for cortisol concentration between the two techniques.

Using a male sample, Rohleder, Wolf, Maldonado, and Kirschbaum (2006) reported a significantly higher volume of saliva being collected using the passive drool method compared to using SOS. Perhaps, then, the finding of a significant interaction between collection method and gender on flow rate should not be completely unexpected. Indeed, this interpretation would support the graphical representation of the interaction (Figure 10), which shows that males produce higher volumes of saliva using the cryovial relative to the SOS. However, comprehensive further investigation showed that neither the gender (i.e. cryovial vs. SOS comparison for either male or female participants) nor technique (i.e. female vs. male comparison for either cryovial or SOS) comparison was independently significant. Therefore, it is suggested that the significant interaction emerged only when all data is considered together due to opposite but very slight patterns of response. It is suggested, also, that the significant interaction might also account for the weaker relationship between the two techniques for flow rate (r = .29) compared to the rest of the comparative correlations

(cortisol: r = 78; sAA activity: r = .83; sAA secretion r = .70) and, consequently, for the significantly different correlation coefficients between flow rate compared with other biomarkers.

It is interesting that, while a significant interaction between gender and collection method on flow rate emerged, no such variability was found in the sAA activity data. This can potentially be considered to provide inadvertent support for Rohleder et al.'s (2006) proposition that sAA is independent to flow rate. If proved to be accurate, such an inference would serve to quell the concerns of Bosch, Veerman, de Geus, and Proctor (2011) regarding the stimulation of flow rate when collecting saliva using absorbent swabs. However, it must again be noted that the origin of the significant interaction in the flow rate data was not successfully isolated; therefore at this stage such conjecture must be drawn with caution. For this reason, and until there is further clarification on the matter, future research should continue to include efforts to collect unstimulated saliva and monitor interference by flow rate.

Since the debate regarding the relationship between sAA and flow rate seems far from settled, it is unsurprising that studies tend to opt to report the output (secretion) measure. In the current study, a highly significant correlation was found between sAA activity and secretion for each of the collection methods (cryovial r = .89; SOS r = .90). For both these reasons, future studies within this thesis will focus solely on the secretion measure and omit reporting details relating to flow rate and sAA activity unless preliminary investigations unearth particularly interesting or contradictory results.

The finding that cortisol concentration is significantly higher in saliva collected through SOS compared to saliva collected with the passive drool method is unanticipated. Prior research investigating the influence of a cotton swab (Salivette or a dental roll) found

cortisol to be one of the few analytes unaffected by collection method (Shirtcliff, Granger, Schwartz, & Curran, 2001). However, the strong positive relationship between cortisol assessed through the two collection methods (r = .78) suggests that both methods should be reliable in ascertaining individual responses to, for example, a stressful procedure assuming the collection method remains constant throughout the study. Cortisol enters the saliva from the bloodstream by diffusing through the cellular membranes rather than being released into the saliva through the salivary glands, like sAA (Vining, McGinley, & Symons, 1983). By being positioned under the tongue, as recommended by the manufacturer to be the prime location for the collection of salivary cortisol, there is maximum contact with areas of cellular membrane within the mouth so perhaps it seems logical that cortisol concentration is higher using this method relative to when 'whole' saliva is collected through passive drool.

In sum, it seems that the use of SOS to collect saliva has little to no interference on physiological biomarkers of the stress response relative to the more established passive drool. Salimetrics (2011) claim their product to be the industry standard in saliva collection. Here, we have found that cortisol is found at greater levels when measured in saliva that was taken using SOS relative to passive drool though the potential reasons, largely relating to positioning of the swab in the mouth, have been discussed. Of practical significance, samples that have been expressed from SOS tend to be much easier to work with relative to passively drooled saliva. Further, expressed samples contain less mucins, which can make saliva stringy and appear tacky. As mentioned earlier, this method does also enable a far more optimised utility based on sample volume, which is always going to be a significant attraction for researchers. For these reasons future studies included in this research will aim to collect saliva with the aid of SOS rather than through passive drool. As there is no research investigating whether this method requires a practice sample, and to prevent straying too much from the thesis' primary research question, future studies in this thesis will always give

participants a practice sample prior to the collection of any baseline saliva. The main reason for this is due to the argument presented in Study 2, which claimed that practice effects might be caused by social embarrassment rather than them completing the process incorrectly, therefore the same argument might hold true with SOS collection.

6.0 CHAPTER SIX: STUDY FOUR

The OCam study: An investigation into the predictive capacity for natural cognitive biases to determine psychophysiological reactions to an ostracism stressor

Incorporating the findings from Studies 1-3, this study aims to further address one of the main areas of investigation of this thesis; looking at the influence of naturally occurring cognitive biases on the psychophysiological stress response. Study 1 aimed to elicit a physiological stress response to a social rejection laboratory stressor based on a previous study that had successfully utilised the task (Blackhart, Eckel, & Tice, 2007). However, cortisol was found to decrease in response to social rejection, and sAA activity was found to decrease following both social rejection and the comparison condition, social inclusion. It was argued that the sAA patterns of response might be unreliable owing to an opposite response being found in flow rate and an absence of any change in sAA secretion. The limited literature generally suggests that sAA activity is independent of flow rate (Rohleder, Wolf, Maldonado, & Kirschbaum, 2006). However, the patterns of response (i.e. sAA activity and flow rate) made it difficult to distinguish what effects were due to the social manipulation and what were due to limitations associated with collection methods.

As this research is using saliva samples as the only index of physiological responses to laboratory stressors and cognitive bias, it is clearly of high importance that samples are of a reliable nature. For this reason, Studies 2 and 3 explored some basic principles surrounding the process of collecting saliva samples in research studies; whether research protocols should stipulate that participants practice the method of saliva donation prior to providing a baseline sample (Study 2), and which of two commonly used collection methods seemed to relay the most reliable and readily utilised sample (Study 3). Results from Study 2 were inconclusive, with some interesting patterns of response suggesting that research that employs female participants specifically might benefit from providing a practice sample. Study 3 demonstrated that giving a sample either through a passive drool method or with the aid of an absorbent swab (Salimetrics Oral Swab) yield similar results. Although cortisol was found in greater amounts in SOS samples, this was thought to be due to the location of the

swab within the mouth being more optimal for absorption of cortisol-rich saliva. In spite of this difference, significant correlations were still found between the two methods for cortisol concentration and sAA activity and secretion. Further, all sAA activity was found to have a highly significant relationship with sAA secretion, which led to the decision to focus only on output (or secretion) data for future analysis.

In contrast to the findings of Study 1, Zadro, Williams, and Richardson's (2004) work into social rejection suggests that the mere insinuation of rejection should be sufficient in producing a powerful emotional response. In line with early theories (e.g. Kune, 1992), such intimations do seem to suggest that people have somehow become evolved to be sensitively attuned to the prospect of social rejection. Williams (1997, 2001) and Williams and Zadro (2005) proposed a need-threat model of social rejection or ostracism that focused on the fulfilment of four basic needs; the senses of belonging, feeling in control, maintaining self-esteem, and satisfaction of having a meaningful existence. When these needs are threatened in a short-term manner, Williams proposed that individuals tend to change their behaviour in a direct effort to restore fulfilment. For example, Williams and Sommer (1997) found that acute rejection led to female participants working harder on ensuing group tasks relative to participants who did not experience rejection. Alternatively, Williams, Cheung, and Choi (2000) noted that rejected participants became submissive to group judgements that were deemed incorrect. Both these findings can be interpreted as reasonable prosocial attempts to gain favourable evaluation in an attempt to replenish satisfaction of the threatened needs. Again, this appears to suggest that, in spite of the failed efforts of Study 1, the area of social exclusion does appear to still show great potential for acting as a stressor, as people seem responsive to it (when successfully implied).

In a review of the recent literature on social rejection, Williams (2007) noted the tendency for researchers to refer to the concepts of rejection, exclusion, and ostracism

interchangeably. While no attempts have been made to empirically compare the differences between how these concepts affect an individual, it is possible to partially separate them based on their methods of induction. For example, Williams speculated that ostracism is more of an implicit notion whereby actions and behaviour infer intentions, with the impression of ostracism often developing over time rather than being an immediately obvious entity. An example of a popular laboratory ostracism paradigm is the participation in a computer game, Cyberball (Williams et al., 2000), during which participants are informed they are playing with either two computer or two human players. Participants are told to randomly throw and catch a virtual ball between their group. Ostracised participants are passed the ball very few times after which they are virtually ignored by the remaining "participants". The aim of this action is to induce feelings of being 'left out' even though no person has specifically declared any preference away from them. Alternatively, Williams claimed social rejection and exclusion refer to situations of isolation following an interaction. While social rejection is thought to occur following explicit information that communicates intentions to exclude someone, social exclusion does not necessarily follow from these explicit declarations (Twenge, Baumeister, Tice, & Stucke, 2001). For example, social rejection might refer to a group of friends telling a person they are not invited to a social occasion. Alternatively, social exclusion would refer to the excluded person simply not being informed of the event.

According to Williams' (2007) classification, Blackhart et al.'s (2007) task (and the task used in Study 1) used a social rejection paradigm, during which participants received feedback following a 'get-acquainted' session that specifically stated that no other participant wanted to work with them on an upcoming task. While both could occur during everyday life, for example by being turned down for a job (social rejection) or being ignored by a loved one following an argument (ostracism), it seems that incidences of ostracism might generally be more personal (e.g. giving a loved one the "silent treatment"). Further, ostracism is argued to

occur in a more public setting relative to exclusion and rejection. Therefore, in consideration of the recommendations made in Study 1 regarding the need for a more public feel to the manipulation of stress, the current study will proceed to adopt an ostracism paradigm to induce stress.

Online ostracism paradigms, such as Cyberball, do seem to have successfully induced the desired psychological response in several studies (e.g. Zoller, Maroof, Weik, & Deinzer, 2010; Williams et al., 2000; Zadro, Boland, & Richardson, 2006) and would arguably present more of a public feel to the social manipulation. However, the task still relies heavily on the perception of ostracism through either feedback or the behaviour of an inanimate object that participants are told is being controlled via another person. In consideration of the difficulty in finding a task considered to be stressful enough to elicit a cortisol response (e.g. Dickerson & Kemeny, 2004), it seems prudent to ensure the task used in the present study is both as believable and effective as possible.

Several studies have used a more public setting to induce ostracism through the use of confederates who act as participants and proceed to ostracise the real participant in much the same way as in the online environments (e.g. Stroud, Salovey, & Epel, 2002). Such an arrangement does serve to create a highly ecologically valid environment and one that is more readily credible. However the use of confederates is not without its shortcomings, primarily requiring a lot of time and effort (and money to compensate confederate's time) but also reducing the degree of control over keeping each experience the same.

Recent work by Goodacre and Zadro (2010) has sought to overcome the inadequacies mentioned above and combine the advantages of ostracism by using "real" people while maintaining the control that standardised tasks offer. This has been achieved through the development of a simulated online web chat task where participants think they are talking in

real time but are actually talking to pre-recorded videos of two confederate participants and a confederate researcher acting in a certain way. This task, termed the Ostracism-Camera (OCam), manages to capture both the face-to-face complexities that typical online tasks neglect whilst exposing participants to a more reliable and controlled task. The task begins by the (real) researcher using an artificial connection page to appear to link in to the web conference, which serves to create the illusion of real-time interaction. Based on specifically rehearsed timings, the (real) researcher then recites a script to appear to be involved in a dialogue with the other (pre-recorded) researcher. For example, the researchers appear to discuss the volume of the conference and, seemingly on request, the (pre-recorded) participants change their positions slightly. The (real and pre-recorded) participants are then instructed that each will deliver a two minute prepared speech relating to light and positive topics, such as hobbies. Each of the pre-recorded confederates present a talk followed by the real participant. Three variants of the video exist, which differ only in their apparent reaction to the (real) participant's presentation. In one version, a neutral video, confederate participants appear to sit politely but do not react in any overly positive or negative manner. An alternate, social ostracism, video features the confederate participants appear to disengage during the (real) participant's speech and chat amongst themselves, seeming to completely ignore the participant. The third version is aimed towards social inclusion, during which the confederate participants display positive behaviour, such as nodding, smiling, and leaning in towards the camera during the (real) participant's speech.

As the original task was developed in Australia (Goodacre & Zadro, 2010), an English version based on the same scripts was developed and tested at the Cognition and Brain Sciences Unit, Cambridge, by Drs. Dunn and Brodbeck. This version was used in the present study and is slightly adapted from the Australian original following a pilot study revealing English audiences to be largely doubtful of the original videos' authenticity. The

actor's responses were considered a little theatrical and so facial expressions and social cues were toned down slightly. For example, in the original ostracism video, at the turn of the genuine participant to present a pre-prepared speech, the confederate participants immediately disengaged and started a conversation between themselves. In the modified version, the confederate participants appeared to pay some attention for 30 seconds before appearing to disengage from the speech. Critically, while the behaviour of the confederates was changed to appear more believable, the manipulation remained as effective as it had been with an Australian audience.

In contrast to Goodacre and Zadro's (2010) original study, which categorised participants into either an ostracism or inclusion group after which participants took part in only one staged conference, the present study required participants to take part in two staged conferences; a neutral conference followed by either an inclusion or ostracism conference. This modification to the original procedure was made in consideration of the stressful nature of presenting in front of a group. Indeed, this type of task is often used in isolation as a form of stressor (e.g. the TSST; Kirschbaum, Pirke, & Hellhammer, 1993). Through this amendment, it was hoped that the stress induced by social ostracism could be determined over and above the stress induced merely by presenting a speech in front of an audience.

Study 1 set out to establish a stressor paradigm that was capable of eliciting reliable physiological responses. This was thought to be a necessary measure prior to introducing a measure of bias to investigate the relationship further. However, evidence has recently surfaced that suggests this might not be the optimal method of pursuing such an investigation. Fox, Cahill, and Zougkou (2010) showed how an individual's natural attentional bias was influential in their subsequent response to a stressor task. Specifically, individuals with a more negative attentional bias were found to be more susceptible to suffering the ill-effects of stress, such as anxiety. Moreover, the predictive power of these biases was found to provide a

better indication than more typically considered markers, such as trait anxiety or neuroticism (Fox et al., 2010). With these findings in mind, a more practical manner of investigating the relationship between bias and psychophysiological vulnerability to stress seems to include a measure of bias regardless of whether or not a reliable stress paradigm has been established. Critically, Fox et al. further imply that failing to account for bias might create unexplained noise in stress-response data. Applying these findings to Study 1 might provide a reasonable explanation for the absence of any effects of the social rejection task; noise in the data. Alternatively, in consideration of the robust link between anxiety and bias strength (for a review, see Ouimet, Gawronski, & Dozois, 2009), and recalling the fact that highly anxious individuals were excluded from Study 1, it might be reasonable to assume that the sample from Study 1 did not have a strong negative bias. Therefore, it is possible that the participant sample was somewhat resilient to the effects of social rejection.

A range of studies have demonstrated that cognitive bias does appear to afford some degree of stress resilience. These studies tend to adopt one of two designs; (1) mapping natural bias to prospective reactions to stressful events (e.g. Pury, 2002; MacLeod & Hagan, 1992; van den Hout, Tenny, Huygens, Merckelbach, & Kindt, 1995), or (2) manipulating bias through CBM techniques and measuring subsequent responses to stressors (e.g. See, MacLeod, & Bridle, 2009; Wilson, MacLeod, Mathews & Rutherford, 2006). Whilst promising, the literature to date has heavily relied on measures of subjective self-report to assess these influences. For this reason, it remained difficult to rule out the presence of demand effects. However, with positive effects of CBM having recently been shown using double-blind placebo-controlled studies (e.g. Amir et al., 2009; Schmidt, Richey, Buckner, & Timpano, 2009), these alternate attributions can start to be discarded. This is further strengthened by studies that show the effects using physiological markers for stress (e.g. Dandeneau, Baldwin, Baccus, Sakellaropoulo, & Pruessner, 2007; Fox et al., 2010). In

acknowledgement of this, the present study included tests of attentional and interpretive bias prior to the OCam conferences. These measures were designed to assess the predictive capacity of bias on emotional and physiological reactivity to stress in line with Fox et al.'s work.

The overall objective of Study 4 is to induce a biological and psychological response to stress using a social ostracism task, and measure the capacity for natural attentional and interpretive cognitive biases to predict the magnitude of physiological (primary aim) and psychological (secondary aim) responses. It is hypothesised that the process of being ostracised (relative to social inclusion) will lead to a significantly higher reporting of feelings of rejection and a significant reduction in the fulfilment of primary needs. Group allocation and bias measures are hypothesised to significantly predict changes in stress, positive and negative affect, cortisol concentration, and sAA secretion following OCam 2 (the socially manipulative video). Specifically, participants undergoing social ostracism are predicted to show a significant psychological and physiological stress response (e.g. increased reporting of stress, increases in cortisol). This response is hypothesised to be moderated by cognitive bias, with stronger negative biases linked with a larger stress response. Finally, this bias-stress response relationship is only predicted to be evident in participants currently undergoing stress, and is therefore predicted to be entirely absent in participants assigned to the social inclusion condition.

6.1 Method

6.1.1 Design

This study adopted a regressional design. Interpretive or attentional bias (within subjects; continuous variable) was entered with group (between subjects; dichotomous variable: ostracism, inclusion) to predict reactivity in dependent variables. The dependent variables were participants' levels of cortisol concentration, sAA secretion rate, reported stress, reported positive affect, and reported negative affect. Measures of social anxiety, rejection sensitivity, trait depression and anxiety, chronic stress and distress, and personality were also taken to assess potential confounding influences on ostracism and stress vulnerability (see Figure 11).

6.1.2 Participants

Staff and students from Anglia Ruskin University were sent details of the study via an all-staff/student email system. Only female participants were recruited in keeping with Study 1 on the basis that social stressors show evidence of being more effective for females (e.g. Stroud, Salovey, & Epel, 2002) and in light of findings from Study 2 that suggests differential physiological patterns between male and female participants. Those interested in taking part contacted the researcher via email to receive further detailed information. Ninety-one participants aged between 18 and 48 years (M = 24.00, SD = 6.57) were chosen following screening for high anxiety using the Spielberger Trait Anxiety inventory (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983; cut off at 60). As a group, mean participant levels of trait anxiety were 39.31 (SD = 9.01). All participants reported being fluent in English, as specified by inclusion criteria.

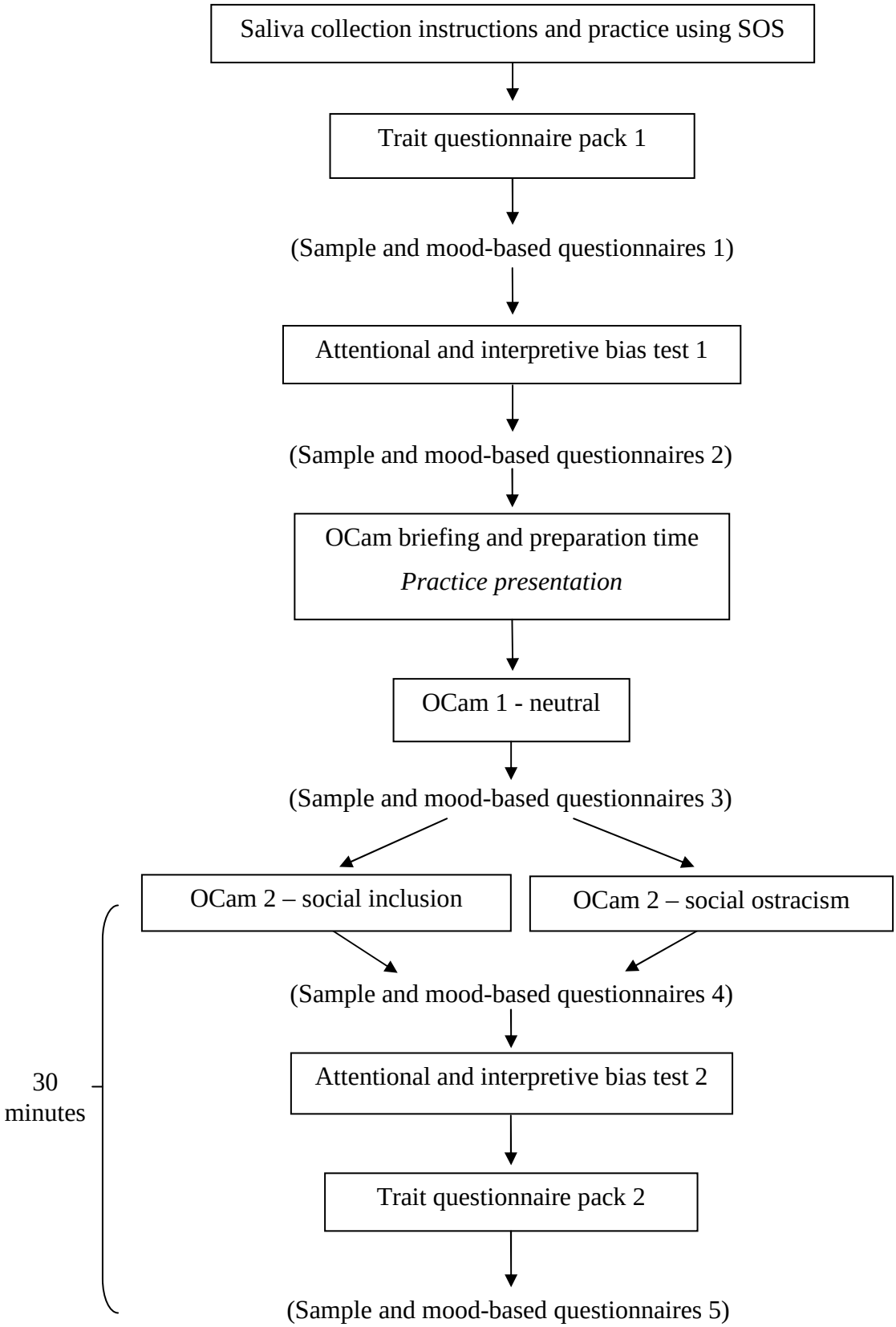

Figure 11. Overview of Study 4's experimental design

6.1.3 Materials

Psychological measures. ***Trait questionnaire pack one***. Participants completed the Depression Anxiety Stress Scale (DASS; Lovibond & Lovibond, 1995b) and Positive and Negative Affect Scale (PANAS-trait; Watson, Clark, & Tellegen, 1988). Further details of these scales can be found in Studies 1 and 2 respectively.

Fear of Negative Evaluation Scale (FNE; Watson & Friend, 1969). The FNE was designed by Watson and Friend (1969) to measure cognitive symptoms of social anxiety. The scale consists of 30 self-report items for which participants indicate how characteristic each item is of them on a forced choice (true/false) basis. Examples of items include *"If someone is evaluating me I tend to expect the worst"* and *"Other people's opinions of me do not bother me"*. Scores on the FNE have been shown to correlate well with alternate measures of social anxiety, such as the Taylor Manifest Anxiety Scale (TMAS; Taylor, 1953) (Watson & Friend, 1969). Responses that indicate social anxiety (e.g. I *do* expect the worst of someone who evaluates me, or implying that other people's opinions *do* bother me) are summed to produce a score out of 30. It has been suggested that a score lower than 12 indicates someone low in social anxiety, and scores above 21 indicate high social anxiety (Watson & Friend, 1969). The authors also report good test-retest reliability (.78-.94), and internal consistency (α = .94-.98).

Adult Rejection Sensitivity Questionnaire (A-RSQ; Downey & Feldman, 1996). The A-RSQ is composed of nine hypothetical interpersonal situations that relate to significant others, for which rejection remains a potential outcome. For each scenario, participants are required to rate on separate 6-point Likert scales a) how much anxiety would be caused by the situation and b) how likely they think the situation would resolve itself in rejection. For example, a scenario might be *"You ask your parents or other family members to come to an*

occasion important to you", for which participants have to provide a rating for the statements "*How concerned or anxious would you be over whether or not they would want to come?"* from 1 (*very unconcerned*) to 6 (*very concerned*), and *"I would expect that they would want to come"* from 1 (*very unlikely*) to 6 (*very likely*). Rejection sensitivity scores are calculated by multiplying reported anxiety together with reported rejection likelihood and then averaging the nine outcomes. The scale demonstrates good internal consistency ($\alpha = .86$; Berenson et al., 2009), good test-retest reliability ($r = .83$) and correlates well with the Social Avoidance and Distress Scale (SADS; Watson & Friend, 1969) ($r = .41$) and the Interpersonal Sensitivity Scale (ISS; Boyce & Parker, 1989) ($r = .48$) (Downey & Feldman, 1996).

State questionnaire pack. Each time participants gave a saliva sample they completed the Positive and Negative Affect Scale (PANAS-state; Watson, Clark, & Tellegen, 1988) and the Stress Arousal Checklist (SACL; Mackay, Cox, Burrows, & Lazzerini, 1978) as outlined in Studies 1 and 2 respectively. As with previous research (e.g. Downey & Feldman, 1996), five adjectives that depict emotions brought about by ostracism were added in a random fashion to the 20-item PANAS-state list. These were: *discouraged, unaccepted, rejected, hurt,* and *disliked.* After both videos, at saliva sample time points 3 and 4, participants additionally completed a 12-item scale designed to assess the impact of feelings of ostracism on four primary need measures (feelings of belonging, self-esteem, meaningful existence, and control; Williams et al., 2002). These items have been used in previous studies investigating social exclusion (e.g. Gonsalkorale & Williams, 2007; Williams, Cheung, & Choi, 2000; Zadro, Williams, & Richardson, 2004).

Trait questionnaire pack two. This pack included a questionnaire consisting of demographic and health-related questions detailing age, alcoholic consumption, time last eaten/drank, recent ill-health, medication, and oral health. Five free-response questions were also included in the pack designed to aid the cover story, which were: "*How easy did you feel*

it was to form new relationships in the web-chat?", *"What do you feel are the positives and negatives about interacting online?"*, *"In your opinion, how does interacting online compare to interacting face-to-face?"*, *"What were your impressions of the individuals in the web-chat?"*, and *"Do you think that the online nature of the chats affected these impressions?"*. Other questionnaires in this pack included the General Health Questionnaire (GHQ-28; Goldberg, 1972; Goldberg & Williams, 1988), the Perceived Stress Scale (PSS-10; Cohen, Kamarck, & Mermelstein, 1983) and a personality questionnaire derived from the International Personality Item Pool (Goldberg et al., 2006), details of which can be found in Study 1.

Manipulation check questionnaire. At the end of the study, before the debrief, participants completed a brief questionnaire asking them questions about how they perceived the study. This, in addition to the five questions asked in the second questionnaire pack, was designed to measure whether participants had noticed any hidden agenda in the study, and served to identify possible exclusions.

Saliva collection. Samples were taken at six different intervals (including a practice) during each session. Participants completed a practice sample soon after arrival into the study. Two baseline samples (Samples 1 and 2) were collected as a considerable amount of time (approximately 50 minutes) that had passed between them both, allowing participants' entering state to be assessed but also as a representative baseline measure. These samples were taken after participants finished the first questionnaire pack and after participants had finished the first of the attentional and interpretive bias tasks. Samples 3 and 4 were collected after each of the OCam videos, and sample 5 was collected 30 minutes after the second OCam video.

For each sample, participants were asked to place a swab (SOS, Salimetrics LLC, US) underneath their tongue for a period of two minutes. Participants were instructed to swallow to clear their mouth of saliva and debris before placing the swab in their mouth and were asked not to chew on or suck the swab. Samples were tracked through the study using a tracking sheet that noted unique barcode details of each tube. Samples were stored at -80°C until required for analysis, and were assayed for levels of sAA and cortisol. Details of the analysis procedures are included elsewhere (see Chapter two).

O-Cam videos. Participants were informed that they would be taking part in two web conference chats with other groups of participants at the University of East Anglia, Norwich. Instructions stated that each conference would consist of three participants in total and that each participant would be required to give a two-minute presentation on any topic that was of a positive or neutral nature. Participants were provided with a short list of ideas of topics to talk about (e.g. recent holidays, career hopes, hobbies, etc.), and given time (approximately 5 minutes) to organise some notes and practice with the researcher before the conference began.

In reality, during this part of the study participants watched pre-recorded videos of actors who appeared to be participating in the task. The videos were recorded at the Cognition and Brain Sciences Unit (CBSU) in Cambridge, and were used with permission of the creators (Drs. Dunn and Brodbeck). Videos were integrated into Visual Basic software, which included an imitation connection screen to aid in the illusion of chatting via an internet connection. At the beginning of each of the two videos, the researcher appeared to share a brief chat with the actor-researcher on the video (actually spoken off a script according to strictly rehearsed timings) to further convince participants of the videos seemingly live nature (see Figure 12). After this staged introduction, the researcher left the room and the participant sat through each of the other two actor's (one male, one female) 2 minute presentations

before giving one herself. The first video appeared to be stopped by the (real) researcher entering the room and cancelling the connection. Again, this was faked, with the researcher actually pressing a inconsequential key sequence on a QWERTY keyboard in time with the video running time expiring.

Figure 12. The starting scene of a neutral O-Cam video with a male virtual researcher.

For the second of the videos, participants either watched a video designed to make them feel socially ostracised or socially included. During the social ostracism video, the participant sat through the actors' (one male, one female) presentations (totalling around four minutes) as before but when it came to their own two-minute talk the actors were seen to tire of the participant and start to talk amongst themselves. In contrast, during the social inclusion video, the actors changed their body language to appear to show great interest in the participant's presentation (for example, by leaning in and smiling/nodding lots). Both videos ended with the (actor) researcher entering the room (in the video) to inform everyone that time had

expired. In the social inclusion video, the actors proceeded to give positive feedback about the task and the participant.

Bias tests. ***Interpretive bias.*** To test interpretive bias, participants completed the recognition test (Mathews & Mackintosh, 2000) that was presented on a computer screen using E-Prime software (Schneider, Eschman, & Zuccolotto, 2002). Participants were presented with 10 scenarios that were preceded by a title and were presented one line at a time. Each scenario remained relatively ambiguous in terms of whether it portrayed a positive or a negative situation. For example, *"Changing the return date on your coach ticket"* (title) *"You bought a coach ticket a while ago to visit a friend." "You now would like to stay an extra day with them but are unsure about the company policies." "You ring the customer service number to change the return date." "You can tell by the operator's tone of voice what they think about your request."* After each scenario, participants have to answer a simple yes/no comprehension question to ensure they had properly understood the situation, e.g. *"Have you decided to change the date of your return coach ticket?"*. After the tenth scenario, participants were required to recall the scenarios through presentation of the title alone, and rate four sentences according to how similar they were to their interpreted recollection of the situation. The four sentences consist of one positive interpretation (e.g. *"The operator seems friendly and sympathetic to your needs"*), one negative interpretation (e.g. *"The operator seems annoyed by your request"*), one positive foil interpretation (e.g. *"The operator politely asks you whether you would like to take advantage of a special offer"*), and one negative foil interpretation (e.g. *"The operator says that the coach you have booked has been cancelled"*). Foil interpretations were included as a control measure to test whether participants specifically recalled the target-related interpretation or just a generally positive or negative situation.

Attentional bias. To measure attentional bias, participants were required to complete a visual probe task (Macleod, Mathews, & Tata, 1986), which included a series of trials for which participants had to respond to probes that were displayed behind neutrally or negatively valenced words. Negative words were related to general (e.g. "inadequate") or sensation (e.g. "ashamed") meanings. Each trial started with a fixation cross being displayed in the middle of the computer screen. After 500ms, this disappeared and was replaced by two words above and below where the fixation cross had been. One of the words was always neutral in valence whilst the other was always negatively valenced. After 500ms, the two words disappeared and an arrow head target probe was displayed in place of one of the two words. Participants were required to indicate whether the arrowhead was pointing to the left ("<") or the right (">") by pressing either the *z* or the *m* letter key on a QWERTY keyboard (which are located on the bottom left and right hand of the keyboard respectively).

The task was presented on a computer using E-Prime Software (Schneider et al., 2002), and was composed of 8 practice trials and 160 test trials split into three sections (54 trials, 53 trials, 53 trials). Between each section participants were given a break, the duration of which they decided. Two buffer trials preceded each of the three sections, which were not included in the analysis. A list containing 20 words was repeated 8 times so that every possible probe-word/word-location combination was used twice (i.e. probe behind neutral word at top of screen and bottom of screen, and probe behind negative word at top of screen and bottom of screen).

Condition assignment. Participants were sorted into conditions (ostracism, inclusion) using a counterbalancing technique. Both the ostracism and the social inclusion conditions had two possible orders of OCam presentation: video 1 with a male researcher followed by video 2 with a female researcher, or video 1 with a female researcher followed by video 2

with a male researcher. Participants were assigned a condition according to the following four-session schedule on a first come first served basis:

1. Neutral video 1 with a female researcher followed by social inclusion video 2 with a male researcher
2. Neutral video 1 with a male researcher followed by social inclusion video 2 with a female researcher
3. Neutral video 1 with a female researcher followed by social ostracism video 2 with a male researcher
4. Neutral video 1 with a male researcher followed by social ostracism video 2 with a female researcher

The male and female actor-researchers worked with a fixed (but different to each other) set of male and female actor-participants, both sets of which were used to socially reject or socially include the participant according to the counterbalancing schedule above.

6.1.4 Procedure

Ethical approval was obtained from the Research Ethics Subcommittee, Anglia Ruskin University. All testing sessions were run on weekdays between the hours of 10am-6pm on-campus at Anglia Ruskin University in Cambridge. Sessions lasted 2-2.5 hours and participants were recompensed with £12 for their effort and time. Participants were instructed not to eat, drink (other than water), or smoke for 30 minutes leading up to the study, and not to undertake vigorous exercise for 90 minutes prior to the study. Participants were first given an information sheet before being verbally briefed on the study and asked to sign a consent form. Participants were then given instructions on how to give a saliva sample and were afforded one practice. Following this, participants were asked to complete the first

questionnaire pack (trait questionnaire pack one) before giving their first non-practice sample. During the two minutes taken to give a sample, participants completed the first copies of the state questionnaires. Following this, participants completed an attentional and interpretive bias task and then gave their second sample and completed a second series of state questionnaires. Participants then received a briefing detailing the nature of the live web conferences (videos) before spending a couple of minutes gathering notes about what they wanted to say. Once prepared, participants were timed for two minutes performing a practice presentation with the researcher before moving on to the two web conference videos. After each video, participants gave a saliva sample and completed the state questionnaires. Next, participants completed a second attentional and interpretive bias tests (a filler task), and the second questionnaire pack (trait questionnaire pack two), before giving their final saliva sample. Participants then completed the manipulation check questionnaire before being fully verbally debriefed and given a written debrief sheet. Following debrief, participants were asked to re-consent to their data being used in this study. Participants were paid and offered the opportunity to take part in a positive mood induction task, which involved noting down three positive life events/experiences that had or were happening to the participant whilst listening to a favoured piece of music.

6.1.5 Data Analysis Plan

Hypothesis 1. To assess the effects of the web-conference task (OCam 1 - neutral) and whether the social manipulation element (social ostracism vs. social inclusion; OCam 2) had been successful, a series of repeated measure ANOVAs were conducted on reported levels of rejection (as measured through the added adjectives on the PANAS), and the measures of primary needs (belonging, control, self-esteem, and meaningful existence).

Hypothesis 2. To test the main hypotheses regarding the influence of cognitive bias on the psychological and physiological effects of ostracism as induced through the O-Cam, a series of moderated regression analyses were performed. Dependent variables were percentage change scores for reported stress (as measured through the SACL), positive and negative affect (as measured through the PANAS), sAA secretion, and cortisol concentration. Percentage change scores were calculated by taking the two measures of interest, for example reported stress at baseline 2 (SACL 2) and reported stress following the second O-Cam video (SACL 4), and conducting the following calculation:

$$((\text{SACL 4} - \text{SACL 2}) / \text{SACL 2}) \times 100$$

The calculation of percentage change in preference to a delta change score thus produced a measure that was relative to the former measure, which enabled all scores to be included regardless of their initial deviation from the group mean. For acute measures (reported stress, positive and negative affect, and sAA secretion) change scores focused on variation between measures 2-3 (response to the task), 2-4 and 3-4 (response to the social manipulation), and 4-5 (recovery). For cortisol, which takes longer for responses to become apparent (e.g. Kirschbaum et al., 1993), change scores were computed between measures 2-5, 3-5, and 4-5 to capture any response to the social manipulation.

These percentage change scores were then entered (separately) as dependent variables into moderated regression analyses with the dichotomous variable (condition: ostracism, inclusion) and the continuous bias score variable (interpretive bias 1 or attentional bias 1) entered as predictors (step 1). A second step (step 2) included an additional interaction term, which was the computed combination of the relevant bias score (as used in step 1) and condition. This was included in regressions that investigated responses to ostracism to investigate whether any predictive capacities of bias were dependent the presence/absence of

stress. To reduce multicollinearity, the continuous variables (attentional bias 1 and interpretive bias 1) were mean-centred prior to computing the interaction terms (Aiken & West, 1991; Holmbeck, 2002). Significant interaction terms were followed up using the process of simple slope analysis (Holmbeck, 2002).

6.2 Results

6.2.1 Data Exploration

Ten participants' data were removed from the analysis, six for expressing suspicion about the deceptive element to the study (live web interaction), three who experienced technical difficulties during the session (e.g. the software being non-responsive), and one due to insufficient understanding of the English language. Of the remaining 81 participants, 40 were in the social ostracism group and 41 participants were in the socially included group. The data was explored for outliers and to check it met the assumptions for parametric testing. Log transformations were calculated for all data obtained by saliva analysis (sAA secretion and cortisol concentration), as it was found to include several outliers and showed positive skewing. This action was successful in its attempts to normalise the distribution of the data. All analyses were conducted using logged data, however descriptive and graphical representation of the means and measures of variation are presented using unlogged data.

6.2.2 Participant Characteristics

Univariate ANOVAs revealed no significant difference between the social inclusion and social ostracism groups on the FNE, RSQ, GHQ-28, PSS-10, DASS (including all three subscales and aggregate score), or STAI-trait (see Table 11). The two groups also did not differ significantly in terms of entry levels of state stress and negative affect (state and trait). However, trait positive affect was found to be higher for the ostracism group than the social inclusion group. Additionally, participants in the ostracism group reported having significantly higher state positive affect than participants in the social inclusion group on entry to the study (at baseline 1; see Table 11). However, these effects were not apparent at baseline 2, $F(1, 77) = .21$, $p = .65$, $\eta_p^2 < .001$ (ostracism condition: $M = 27.63$, $SD = 7.67$;

inclusion condition: M = 26.76, SD = 9.16), which was used as the primary baseline comparison point (see below).

Table 11

Means and standard deviations for participant trait and entry state characteristics

		Social ostracism		*Social inclusion*			
		Mean	*SD*	*Mean*	*SD*	*F value*	*p value*
FNE	Social anxiety	6.03	4.00	6.51	4.56	.26	.61
RSQ	Rejection sensitivity	8.84	3.28	9.21	3.38	.25	.62
DASS	Depression, anxiety and stress (aggregate)	23.78	15.41	26.77	16.73	.65	.42
GHQ	Distress	46.05	8.69	48.94	11.08	.77	.39
PSS-10	Chronic stress	16.33	4.82	17.18	5.29	.49	.49
STAI	Trait anxiety	38.60	7.60	39.59	10.31	.24	.63
PANAS (trait)	Trait positive affect	36.63	6.45	33.85	5.54	4.19	.04
PANAS (trait)	Trait negative affect	18.97	6.10	20.00	7.07	.47	.50
SACL	Entry state stress	2.85	3.07	3.76	3.30	1.64	.21
PANAS (state)	Entry state positive affect	31.47	7.60	27.66	8.21	4.58	.04
PANAS (state)	Entry state negative affect	13.50	4.64	13.60	4.40	.01	.92

6.2.3 Baseline Sample

A series of 2 (group: social ostracism, social inclusion) x 2 (time of sample: baseline 1, baseline 2) repeated measures ANOVAs revealed that there were no significant main effects of time (all *p* values > .10; see Table 12) or group (all *p* values > .12; see Table 12) between the first and second baseline samples for sAA secretion, feelings of rejection, or reported stress. For these variables, there was also found to be no significant interaction between time and group allocation (all *p* values > .14; see Table 12).

Table 12

Mean (and SD) comparisons for baselines 1-2 and statistical output

	Baseline 1		Baseline 2							
	Mean	*SD*	*Mean*	*SD*	Time Main Effect		Group Main Effect		Group x Time Interaction	
					F	*p*	*F*	*p*	*F*	*p*
Cortisol (µg/dl)	.21	.16	.18	.12	15.39	<.001	.05	.83	.13	.72
sAA secretion (U/min)	14.72	21.20	15.18	17.56	2.80	.10	.20	.65	.27	.61
Reported stress (SACL)	3.31	3.20	3.62	3.67	.82	.37	2.44	.12	.21	.65
Reported rejection (PANAS)	6.38	4.30	5.63	1.52	2.55	.11	.90	.35	.77	.38
Reported positive affect (PANAS)	29.49	8.10	27.18	8.43	27.46	<.001	1.72	.19	10.54	.002
Reported negative affect (PANAS)	13.55	4.49	12.81	3.96	4.06	.05	.20	.65	.65	.42

As can be seen in Table 12, there was a significant main effect of sample time on cortisol concentration; cortisol samples were significantly higher at Baseline 1 relative to Baseline 2. However, there was no significant main effect of condition and no significant interaction between group allocation and time of sample. A significant time main effect was also found for positive and negative affect; levels of both significantly decreased between Baseline 1 and Baseline 2 (see Table 12). No significant main effect of group was revealed for either positive or negative affect. While no significant time x group interaction was identified for negative affect, a significant time x group interaction was identified for positive affect. Further investigation, in the form of post-hoc paired *t*-tests, revealed that for the social ostracism group levels of positive affect fell significantly from a mean of 31.47 (*SD* = 7.60) to 27.63 (*SD* = 7.67), $t(37) = 5.50$, $p < .001$, $d = .50$. For participants in the social inclusion group, there was no significant change in reported positive affect, $t(40) = 1.55$, $p = .13$, $d = .10$ (Baseline 1: $M = 27.66$, $SD = 8.21$; Baseline 2: $M = 26.76$, $SD = 9.16$).

Univariate ANOVAs showed no significant main effect of condition on cortisol, sAA secretion, or reported stress, rejection, positive affect and negative affect at baseline 2. In light of this, and due to it being a better representation owing to the closer proximity in time, baseline 2 (sample 2) was selected as the most suitable baseline sample to compare against successive samples.

6.2.4 Creating Bias Index Scores

Interpretive bias index (IBI) scores. Prior to calculating an IBI score, a series of paired *t*-tests were conducted to distinguish whether participants successfully discriminated between target and foil sentences during the recognition task. Results revealed that participants consistently rated target positive items ($M = 2.19$, $SD = .47$) higher with regards to their recollection of how the sentence matched the original scenario relative to positive foil

items (M = 1.58, SD = .49), $t(80)$ = -15.19, $p < .001$, d = 1.26. Similarly, participants were found to rate negative target items significantly higher (M = 2.27, SD = .51) in comparison to negative foil items (M = 1.40, SD = .34), $t(80)$ = -17.31, $p < .001$, d = 2.02. This confirmed that participants were correctly recalling interpretations of the scenario, rather than making generalised positive or negative associations.

To produce an overall IBI score, individual target ratings for the negative interpretations of sentences were subtracted from target ratings for the positive interpretations of sentences. The resulting IBI score represented an individual's overall tendency to make positive or negative interpretations of ambiguous scenarios (i.e. their natural interpretive bias), with a higher score representing a more positive bias and a more negative score indicating a more negative bias.

Attentional bias index (ABI) scores. Prior to calculating an ABI score, incorrect trials and trials for which participants took less than 200ms or longer than 2000ms to complete were removed from the analysis (as in MacLeod, Rutherford, Campbell, Ebsworthy, & Holker, 2002). This extraction consisted of 2.38% of the total available test data. To produced a single useable attentional bias index score, median time (milliseconds) taken to respond to probes displayed behind the neutral words was subtracted from median time (milliseconds) taken to respond to probes displayed behind the negative words. A higher resulting score indicated a more positive bias, whilst a lower number indicated a more negative bias.

6.2.5 Hypothesis One

Social rejection. A 2 (condition: social ostracism, social inclusion) x 4 (time point: baseline 2, post OCam 1, post OCam 2, and 30 minutes post OCam 2) repeated measures ANOVA was conducted on reported rejection (from PANAS adjectives). A significant main

effect of time was identified, $F(1.65, 126.88) = 28.50$, $p < .001$, $\eta_p^2 = .27$ (Greenhouse Geisser adjusted), that was qualified by a significant interaction between time and condition, $F(1.65, 126.88) = 36.09$, $p < .001$, $\eta_p^2 = .32$. Post-hoc investigation (Bonferroni corrected $\alpha = .017$) of the main effect revealed no change from Baseline 2 until after OCam 1, $t(79) = .38$, $p = .71$, $d = .03$, a significant increase between the two OCam videos, $t(79) = -4.77$, $p < .001$, $d = .70$, followed by a significant decrease from OCam 2 until 30 minutes later, $t(78) = 4.57$, $p < .001$, $d = .55$. As expected, when exploring the interaction, a significant increase in reported rejection was identified only in socially rejected participants immediately after watching the socially rejecting OCam video, $t(38) = -6.44$, $p < .001$, $d = 1.38$. In this group, reported rejection was then found to significantly decrease after 30 minutes from watching the second OCam video, $t(38) = 6.07$, $p < .001$, $d = 1.06$. No other comparisons approached statistical significance in either the social ostracism or social inclusion condition (see Figure 13). Further, univariate ANOVAs showed no significant main effect of condition on reported rejection at baseline 2, after (neutral) OCam 1, or 30 minutes after OCam 2 (all p values > .24). After OCam 2, a significant main effect of condition was evident, $F(1, 79) = 42.23$, $p < .001$, $\eta_p^2 = .35$.

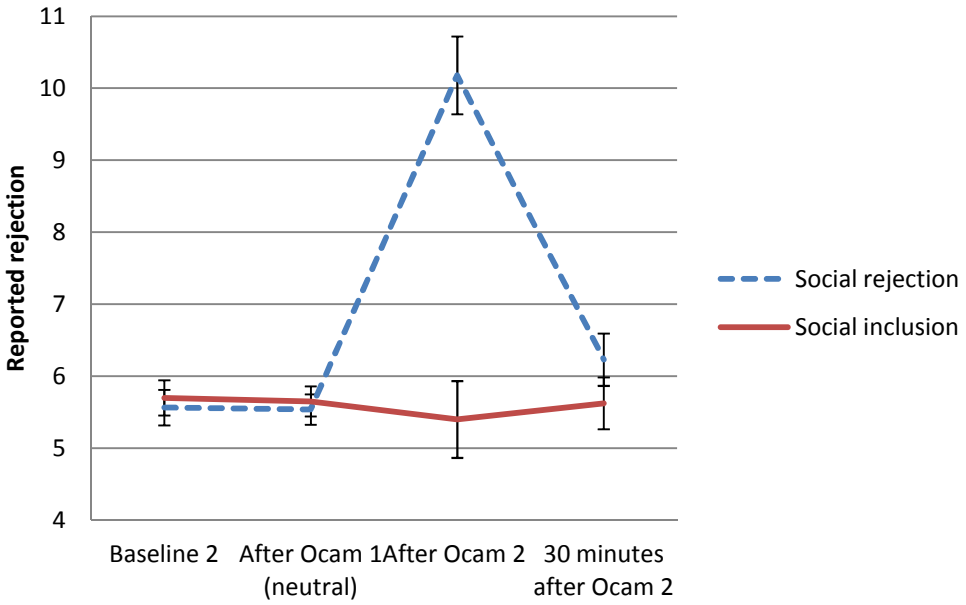

Figure 13. Change in reported rejection throughout the study.

Primary needs. A 2 (between subjects; group: social ostracism, social inclusion) x 2 (within subjects; time point: after [neutral] OCam video 1, after [socially manipulating] OCam video 2) repeated measures ANOVA was conducted on the aggregate scores of the four primary needs variables. While no main effect of time was revealed, $F(1, 71) = 2.88$, $p = .09$, $\eta_p^2 = .04$, a significant interaction was identified between group and time, $F(1, 71) = 45.09$, $p < .001$, $\eta_p^2 = .39$.

Post-hoc testing (Bonferroni corrected $\alpha = .03$) revealed that participants who were socially rejected reported a significant decrease in overall primary needs fulfilment, $t(36) = -5.75$, $p < .001$, $d = 1.23$. Conversely, participants in the social inclusion condition reported a significant increase in primary needs fulfilment, $t(35) = 3.69$, $p = .001$, $d = .69$. Table 13 shows the output of these analyses when run on the subgroups of the primary needs variables. As can be seen, the fulfilment of needs associated with belonging, self-esteem, and having a

meaningful existence all appear to be affected by social ostracism in the hypothesised manner. The need for control, alternatively, is not affected by the social manipulation[7].

Table 13

The relationship between primary need subscales and social manipulation

	Belonging	Self-esteem	Meaningful existence	Control
		REPEATED MEASURES ANOVA		
Main effect of time	$\mathbf{F(1, 74) = 15.49^{**}}$	$F(1, 74) = .02$	$\mathbf{F(1, 75) = 10.48^{*}}$	$F(1, 76) = .08$
Interaction with social manipulation	$\mathbf{F(1, 74) = 71.32, ^{**}}$	$\mathbf{F(1, 74) = 35.86^{**}}$	$\mathbf{F(1, 75) = 45.95^{**}}$	$F(1, 76) = 3.27$
*** $p < .01$ ** $p < .001$**				
		PAIRED *T*-TESTS		
Ostracism only	$\mathbf{t(37) = -7.00^{*}}$	$\mathbf{t(37) = -3.78^{*}}$	$\mathbf{t(38) = -5.57^{*}}$	
	Reduced levels of belonging	*Reduced levels of self-esteem*	*Reduced levels of meaningful existence*	*N/A*
Inclusion only	$\mathbf{t(37) = 4.83^{*}}$	$\mathbf{t(37) = 5.00^{*}}$	$\mathbf{t(37) = 4.25^{*}}$	
	Increased levels of belonging	*Increased levels of self-esteem*	*Increased levels of meaningful existence*	*N/A*
*** $p < .013$ (Bonferroni corrected alpha)**				

Summary. As hypothesised, reported rejection was significantly greater in response to social ostracism relative to social inclusion. Ostracism additionally led to a reduced

[7] This finding was supported by univariate ANOVAs conducted on the aggregate and individual subscale scores comparing the influence of condition prior to and after OCam 2. For all scales there was no main effect of condition at measure 1 (all *p* values > .17), but for the aggregate scale and belonging, self-esteem, and meaningful existence subscales there was a significant main effect of condition (in the predicted direction) at measure 2 (all *p* values < .001). No main effect was apparent for the control subscale at measure 2 ($p = .16$).

fulfilment of individual primary needs, specifically the subscales related to belonging, self-esteem, and having a meaningful existence. Alternatively, in relation to the need to have control, there was no support for the hypothesis.

6.2.6 Hypothesis Two

Attentional Bias. ***Response to neutral task (OCam 1).*** Prior to the moderated regression, simple linear regression analyses were conducted to confirm whether random allocation to groups (ostracism, social inclusion) had been successful. Results found that group allocation and attentional bias did not significant predict changes in sAA secretion, positive affect, or negative affect between measures 2-3 (baseline 2 and after OCam 1; see Table 14). Attentional bias did emerge as a significant unique predictor of changes in negative affect between measures 2 and 3 (p = .04). The absence of predictive capacities of group allocation at this stage confirms that random allocation to conditions took place as, at this point in the study, there were no differences in protocol between the two conditions.

Table 14

Summary of regression analyses testing effects of group allocation and attentional bias.

	Model		R^2	Group allocation		Attentional bias	
				B	*SE*	*B*	*SE*
A	Stress change	2-3	.001	-.03	.53	.004	.02
	Positive affect change	2-3	.03	-.02	.06	-.003	.002
	Negative affect change	2-3	.06	-.03	.05	-.003*****	.002
	sAA change	2-3	.01	.61	.93	.001	.03

Note. A = Response to task

Response to social manipulation. A series of moderated regressions were conducted to assess the predictive capacity of group allocation and cognitive bias on psychological and physiological responses to ostracism/social inclusion. Step 2 further assessed the importance of being in a current state of stress in observing these effects. A significant proportion of the variance in reported stress, positive affect, and negative affect between measures 3-4 (post OCam 1; post OCam 2) and 2-4 (baseline 2; post OCam 2) was accounted for by group allocation and attentional bias on Step 1 (see Table 15). However, for all models, group allocation was found to be the only significant predictor. Additionally, the interaction terms (group x attentional bias; step 2) did not emerge as a significant predictor for any of the dependent variables. No significant amount of variation in sAA secretion (measures 3-4 or 2-4) or cortisol (measures 2-5, 3-5, or 4-5) was explained by group allocation, attentional bias or the interaction term (group allocation x group).

Recovery. A series of moderated regressions were conducted as above to focus on recovery from the task. Group allocation and attentional bias (step 1) accounted for a significant amount of variance in reported stress, positive affect, and negative affect between measures 4-5 (post OCam 2; 30 minutes later), though group allocation emerged as the only significant predictor. Group allocation and attentional bias failed to account for any significant variation in sAA secretion between samples 4-5. No interaction terms (step 2) were significant (see Table 15).

Table 15

Summary of regression analyses testing moderating effects of group allocation and attentional bias.

			Step 1					Step 2	
				Group allocation		Attentional bias			Interaction term (group x bias)	
	Model		R^2	*B*	*SE*	*B*	*SE*	ΔR^2	*B*	*SE*
B	Stress change	3-4	.18**	1.46***	.39	.003	.01	.01	.02	.02
		2-4	.19**	1.73***	.47	-.004	.02	.02	-.04	.03
	Positive affect change	3-4	.31***	-.24***	.04	.001	.001	.001	.001	.003
		2-4	.23***	-.28***	.06	.000	.002	.001	-.001	.004
	Negative affect change	3-4	.26***	.29***	.06	.002	.002	.001	.001	.004
		2-4	.19***	.28***	.07	-.002	.002	.01	-.004	.004
	sAA change	3-4	.05	-.37	.28	.01	.01	.02	-.02	.02
		2-4	.01	.15	.46	.01	.01	.002	.01	.03
	Cortisol change	2-5	.04	.10	.10	.003	.003	.001	.002	.01
		3-5	.03	.000	.08	-.003	.002	.01	.004	.005
		4-5	.05	.04	.06	-.003	.002	.000	.001	.004
C	Stress change	4-5	.12*	-.59**	.19	.000	.01	.003	-.01	.01
	Positive affect change	4-5	.13**	.14**	.05	.002	.002	.02	.004	.003
	Negative affect change	4-5	.17**	-.15***	.04	.000	.001	.001	.001	.003
	sAA change	4-5	.005	.15	.32	-.004	.01	.004	.01	.02

Note. B = Response to social manipulation, C = Recovery; * $p < .05$, ** $p < .01$, *** $p < .001$.

Interpretive Bias. ***Response to neutral task (OCam 1).*** Prior to the moderated regressions, simple linear regression analyses were conducted to determine the effects of participating in the neutral task and to ensure successful random group allocation had taken place. Group allocation and interpretive bias were not found to account for any significant amount of the variance in positive affect, negative affect, or sAA secretion between measures 2-3 (baseline 2; post OCam 1). While the model was not found to be significant for variation in stress, interpretive bias did emerge as a near-significant predictor (p = .05; see Table 16). The absence of any predictive power of group allocation confirms that random group allocation took place.

Table 16

Summary of regression analyses testing effects of group allocation and interpretive bias.

				Group allocation		Interpretive bias	
	Model		R^2	*B*	*SE*	*B*	*SE*
A	Stress change	2-3	.06	.06	.52	.94 *[1]	.47
	Positive affect change	2-3	.01	-.02	.06	-.05	.06
	Negative affect change	2-3	.01	-.04	.05	-.003	.05
	sAA change	2-3	.01	.59	.93	.32	.80

Note. A = Response to task. *[1] p = .052.

Response to social manipulation. Moderated regressions were conducted to determine the predictive capacity of group allocation and cognitive bias. Step 2 assessed whether considering an individual's current state (i.e. stressed or not stressed) was necessary in observing these effects. A significant amount of variance in stress, positive affect, and

negative affect between measures 3-4 and 2-4 was accounted for by group allocation and interpretive bias (step 1; see Table 17). However, group allocation emerged as the only significant predictor in all models. No significant amount of variance in these models was explained by the interaction terms (group x interpretive bias; step 2). No significant amount of variance in sAA secretion (measure 3-4 or 4-5) or cortisol (measures 2-5, 3-5, or 4-5) was explained by group allocation, interpretive bias, or the interaction term.

Recovery. In step 1, a significant amount of variation in stress, positive affect, and negative affect between samples 4-5 was accounted for by group allocation and interpretive bias, though in all instances group allocation emerged as the only unique significant predictor. While group allocation and interpretive bias (step 1) were not found to account for a significant amount of variation in sAA secretion between samples 4-5, interpretive bias did emerge as a trend unique predictor (p = .08). No interaction terms (group x interpretive bias; step 2) were found to account for a significant amount of variance in any of the recovery models.

Table 17

Summary of regression analyses testing moderating effects of group allocation and interpretive bias.

			Step 1					Step 2		
				Group allocation		Interpretive bias			Interaction term (group x bias)	
	Model		R^2	*B*	*SE*	*B*	*SE*	ΔR^2	*B*	*SE*
B	Stress change	3-4	.19**	1.44***	.39	.41	.34	.03	1.04	.70
		2-4	.20**	1.76***	.46	.47	.42	.001	-.26	.86
	Positive affect change	3-4	.30***	-.24***	.04	-.004	.04	.004	.05	.08
		2-4	.23***	-.28***	.06	-.05	.05	.01	.10	.11
	Negative affect change	3-4	.25***	.30***	.06	-.02	.06	.005	-.08	.11
		2-4	.18***	.28***	.07	-.02	.06	.01	.14	.13
	sAA change	3-4	.02	-.36	.29	.07	.25	.02	.59	.51
		2-4	.004	.16	.46	-.15	.41	.003	.38	.83
	Cortisol change	2-5	.02	.11	.10	.04	.09	.01	.17	.20
		3-5	.000	-.01	.08	.01	.07	.02	.14	.15
		4-5	.01	.04	.06	.02	.05	.01	-.08	.11
C	Stress change	4-5	.13**	-.60**	.19	.18	.17	.03	-.49	.34
	Positive affect change	4-5	.11*	.14**	.05	.03	.04	.02	-.12	.09
	Negative affect change	4-5	.18**	-.15***	.04	.03	.04	.003	-.04	.07
	sAA change	4-5	.05	.14	.31	-.49 *[1]	.27	.000	.03	.56

Note. B = Response to social manipulation, C = Recovery; *[1] p = .08, * p < .05, ** p < .01, *** p < .001.

Summary

Group allocation was found to be a significant predictor for variation in reported stress, positive affect, and negative affect between measures 3 (post OCam 1) – 4 (post OCam 2) and 2 (baseline 2) – 4, as well as for variation in these variables between measures 4 – 5 (30 minutes post OCam 2). There were no significant predictors of cortisol or sAA secretion between these time points, though interpretive bias was found to be a trend predictor of variation in sAA secretion between time points 4-5. Group allocation was not found to be a significant predictor of changes in dependent variables between time points 2-3, though attentional bias was found to significantly predict variation in negative affect and interpretive bias was found to be a near significant predictor of changes in stress during this time.

6.3 Discussion

As predicted, participation in a simulated online chat that was staged to induce the sensation of being ostracised successfully produced a subjective stressful response. Ostracised participants reported a significant increase in feelings of rejection and significantly less fulfilled primary needs compared to a simulated online chat that promoted feelings of social inclusion. Specifically, ostracised participants reported significantly less fulfilment of primary needs associated with belonging, self-esteem, and having a meaningful existence while the subgroup of needs associated with being in control was unaffected. Allocation to either a social ostracism or social inclusion condition significantly predicted variation in psychological measures; reported stress and positive and negative affect. However group allocation failed to predict any changes in the physiological measures, thereby suggesting that ostracism was ineffective in producing significant changes in cortisol and sAA. Further, attentional bias did not significantly predict any physiological changes during the study. However, attentional bias did significantly predict changes in negative affect from baseline 2 until after OCam 1 (taken to represent a response to participating in the task). Interpretive bias emerged as a trend predictor of changes in stress from baseline 2 to after OCam 1 (response to the task), and for changes in sAA between the final two samples (recovery from the stressor/task).

Weeks, Heimberg, Rodebaugh, and Norton (2008) argued that social anxiety is composed of a general fear of evaluation (i.e. a fear of both negative *and* positive evaluation). While positive evaluation fears were not measured in this study, negative evaluation fears were using the Fear of Negative Evaluation scale (Watson & Friend, 1969). This construct was not found to significantly vary by condition. Though the two concepts are thought to exist independently, they have been shown to share a strong positive correlation in an undergraduate sample (Weeks et al., 2008; Weeks, Heimberg, & Rodebaugh, 2008). It is

therefore assumed that social anxiety did not act as a confounding factor to responses of ostracism or inclusion in this study.

As with Study 1, the stressor task in the present study (being ostracised) did not elicit any physiological stress response. However, unlike Study 1, here the task was successful in producing an acute psychological response on standardised measures, specifically increases in stress, negative affect, and feelings of rejection and decreases in positive affect and self-reported fulfilment of primary needs. It seems, therefore, that there is some inconsistency between how participants report feeling and their physiological response. In order to further examine this disparity, it seems worth considering how people responded to earlier aspects of the study. As mentioned previously, this task does appear to contain elements of the TSST, notably self presentation in front of an (assumed) audience. In a meta-analyses of 208 studies that aimed to induce a physiological (cortisol) response, Dickerson and Kemeny (2004) concluded that three elements were necessary in order to produce a task-induced physiological response; uncontrollability, motivation to succeed, and threat to the social self. The intended stressor (ostracism) aspect of this study was thought to contain these aspects. Motivation was represented through an individual's natural desire to belong (Baumeister & Leary, 1995), while uncontrollability was illustrated by forcing participants to continue talking for two minutes to an audience (the self-presentation aspect of which contained a potentially socio-evaluative and personal element). However, while the self-presentation aspect might have been considered uncontrollable, Williams et al.'s (2002) fundamental need for control was not found to be significantly affected by the perception of ostracism. Perhaps, then, it could be argued that the first OCam video (neutral response) acted as the stressful task over and above actually being rejected. From a participant's perspective, for example, the first task would still include an element of social evaluation. Post-hoc analysis conducted between baseline 2 and OCam 1 appeared to support this proposition. In all participants, both

reported stress, $t(80) = -2.62$, $p = .01$, $d = .30$, and sAA secretion, $t(74) = -5.30$, $p < .001$, $d = .50$, increased significantly, which suggests that participating in the task itself (i.e. self-presentation to an audience) proved stressful.

The suggested reinterpretation of the stressor discussed above could have considerable ramifications in terms of explaining the apparent discrepancy between physiological and psychological responses to social ostracism. For instance, condition allocation, and therefore social ostracism, failed to predict any changes in sAA in response to the second, socially manipulative, OCam task. It remains possible that the significant rise in sAA secretion in response to the task (i.e. OCam 1) alone acted as a mask to any response to the social ostracism element which, if combined with a neutral non-stressor task, might have been more apparent.

By coincidence, while proving disadvantageous in one outlook, these post hoc findings relating to the effects of the task itself do prove useful when considering another viewpoint. For instance, initial analysis had suggested there had been no physiological response. However, retrospectively finding a significant rise in sAA secretion in response to the task means that changes between the final two samples represented a real recovery from stress. Accordingly, any associations linked to this recovery phase appear instantly more significant. Interpretive bias was found to be a trend predictor of changes in sAA during this time, with a stronger negative bias being indicative of a slower return to baseline. While not appearing influential in physiological *responses* to stress, these results suggest that interpretive bias might instead determine an individual's success in *recovering* from a stressful event.

Partial support for the above argument is given by recent findings from Baert, Casier, and De Raedt (2011), who were able to link the effects of attention modification training to

an individual's physiological recovery from, rather than their response to, stress. Participants who received attention modification training for six days prior to a mock interview showed a significantly faster recovery of heart rate variability compared with participants who completed a control version of the training. While researchers typically focus solely on the stress response, perhaps consideration of both response to and recovery from a stressor might provide a better framework in determining resistance/resilience to stress. Indeed, the Perseverative Cognition hypothesis (Brosschot, Gerin, & Thayer, 2006) argues that it is this capacity to recover from a stressor that serves as a better predictor for stress-related ailments, such as poor health, rather that the magnitude of the initial response.

The absence of significant findings relating to the predictive power of attentional bias is surprising, given that this bias has received considerably more interest in the literature than interpretive bias. Several studies have documented attentional bias as a correlate of an individual's cortisol response to a stressful event (e.g. Dandeneau, Baldwin, Baccus, Sakellaropoulo, & Pruessner, 2007; Pilgrim, Marin, & Lupien, 2010). Fox, Cahill, and Zougkou (2010) extend this with findings that showed how hyper-vigilance to threat predicted cortisol response to stress above and beyond the predictive power of more conventionally considered trait factors, such as anxiety, neuroticism, and extraversion. It is worth noting, though, that the above studies tailored tests of attention to match the genre of stress included. For example, to measure attention, Pilgrim et al. employed a visual probe task that contained words that were specifically selected for their ability to convey emotions depicted with social evaluation. For the stress task, the authors then used a modified version of the TSST that included self-presentation. The current study aimed to measure the influence of a more general measure of attention, with emotive words that pertained to either a generally negative (e.g. negligent) or negative sensation (e.g. suffocating) category. Alternatively, the categories included in the interpretive bias test related more to social

interaction and performance, which might explain both the disparate findings between the two measures of bias included in the present study and also the relationship between attentional bias observed in the present and previous studies.

Three main points can be concluded from the findings of the present study. First, social ostracism created using the OCam paradigm appeared to successfully act as an acute psychological stressor. However, confounding aspects of the task (self-presentation) possibly concealed evidence of ostracism being an acute physiological stressor. For this reason, it would seem unwise to continue with this task in its current form (i.e. with a "neutral" then ostracising video). Second, as argued in a recent paper (Mackintosh, Mathews, Eckstein, & Hoppitt, in prep), it seems that there is a necessity to match bias test material with similar concepts that might be included in the emotional appraisal of the stress task in order for any influences to become visible. Finally, drawing on findings concerning data from interpretive bias tests (which did correspond more to the domain of the stressor), the data suggests that natural biases might additionally play a role in an individual's ability to recover from a stressful event. This implies that future research should consider both response and recovery changes and suggests the relationship between bias and stress vulnerability might not be as clear cut as previously assumed. The next logical step in this research (Study 5) will address the influence of bias training on the stress response/recovery process.

7.0 CHAPTER SEVEN: STUDY FIVE

An investigation into the influence of CBM-I training on the psychophysiological effects of acute stress

There now exists a well established causal link between cognitive bias and anxiety; an individual's inclination to disproportionately focus on threatening material and interpret ambiguity in an overly threatening manner can determine their susceptibility to various anxieties (for a review, see Beard, 2011). The manners in which these biases operate on a physiological level, however, are less well understood. A handful of researchers have started to explore this area, with findings demonstrating influences in the expected direction. For example, Fox, Cahill, and Zougkou (2010) have shown how individuals' unconscious tendency to selectively attend to threatening material determined cortisol responses to acute laboratory stressors delivered four and eight months later. Dandeneau, Baldwin, Baccus, Sakellaropoulo, and Pruessner (2007, experiment 1) also found a significant positive link between cortisol responses to a stressor (rejection task) and attention bias on a visual probe task. Here, cortisol output was greater in participants who were faster to respond to probes that were placed behind pictures of angry or rejecting expressions. In this thesis, Study 4 failed to replicate the suggested links presented above, as changes in cortisol following an acute stressor were not found to be explained by natural biases in attention and interpretation. However, interpretive bias was found to influence the recovery rate of sAA, which has previously not been explored. The present study aims to further the current literature by investigating the influence of positive CBM-I training on the psychological and physiological stress response.

At present, one study exists that explores the influence of bias training on the physiological stress response. Dandeneau et al. (2007, experiment 3b) created a novel bias training program that required participants to locate a picture of a face that depicted a neutral/happy emotion from a 4x4 matrix of faces, of which the remaining 15 pictures depicted angry/rejecting expressions. Compared with a control condition, participants who completed the 'find the happy face' training daily for one week had significantly lower levels

of cortisol and significantly smaller peak cortisol responses over the working day, thereby indicating they had been less affected by the social stress associated with their jobs (telemarketing).

Though in its infancy, the highlighted research offers a great deal of promise for researchers in the field. However, to date, only two studies have investigated the influence of naturally occurring *interpretive* biases on the physiological stress response. In Study 4, interpretive bias was not found to significantly predict physiological (or psychological) reactions to a social ostracism paradigm. Interestingly, however, the measure was indicative of changes in sAA (though not cortisol) after the stressor, implicating a potential influence of interpretive bias on recovery success following stress rather than at the initial response stage. Concurrent with the efforts presented in this thesis aimed at establishing a link between cognitive biases and the stress response, a collaborative investigation explored this link focusing on performance-related stress (Hoppitt, Mackintosh, Randall, & Bristow, under review). Interpretive bias to emotionally ambiguous vignettes was measured using the recognition test. Participants were then exposed to either a stressor or control task in a group setting. The stressor required participants to complete a series of computer tasks that they were told formed a cognitive ability test. The three tasks were presented in a set order and consisted of a number and general memory test and a series of anagrams. Participants were informed the test had been designed so that "average" students performed well, when actually the tests were set to a high level of difficulty to induce feelings of failure. For the control condition, participants were presented with the same instructions and tasks but with the difficulty set at an easy and unchallenging level. Though no main effects of the stressor were evident on the physiological stress response, findings demonstrated a clear link between interpretive bias and the stress response. Participants in the stress condition with a more

negative bias (following a median split) were found to have significantly greater cortisol and sAA responses to the task relative to those with a more positive bias.

Though considerably more research is needed to replicate these findings and clarify any conflicting findings, preliminary research suggests that bias might play a seminal role in how people respond to a stressful event. One possible cause of the discrepancies in the two studies mentioned above could be due to the type of stimuli used in the bias tests/training. For example, in Study 4 (of the present thesis) attention bias was not found to significantly predict any physiological changes in response to a social ostracism challenge. It was noted, however, that the content of the test word lists did not match that of the stressor, with the test stimuli corresponding to generally negative or negative sensation categories while the stressor characterised a social evaluative stressor. Dandeneau et al. (2007, experiment 3a) report similar domain-specific effects in a study during which participants completed an online 'find the happy face' training exercise for five days prior to a final exam. Each day, training was followed by participants answering three questions relating to their appraisal of their exam anxiety. While the training was successful in reducing their anxiety specifically relating to the exam, it had no influence on general levels of stress or anxiety over the training days.

In studies where clear bias effects are evident (e.g. Pilgrim, Marin, & Lupien, 2010), the bias test and stressful challenge tend to encompass similar domains (e.g. social stress). Mackintosh, Mathews, Eckstein, and Hoppitt (in prep) explored these specificity patterns by training participants toward a more positive bias with material that either matched or differed in content to an ensuing stressful task. Findings showed that training effects were only apparent in the response to the stressor when the training was more tailored to the task. These findings imply that biases function at a domain-specific level. This issue has received considerable attention within the field (e.g. Salemink, van den Hout, & Kindt, 2010), though

at present findings are often contradictory. Therefore, as this is not an issue that is to be addressed here, for the purpose of the present study bias training/test material will be tailored specifically to match themes evident in the stressful task.

The main objective of the present study is to investigate the influence of interpretive bias training on the psychological and physiological response to a stressful event. In light of the specificity arguments presented above, and considering no clear bias-stress response links were identified in Study 4, the present study will adopt the more successful stressor paradigm employed in Hoppitt et al. (under review). A further justification for implementing the imitation cognitive ability stressor tasks in the current study arises from the fact that an interpretive training programme has already been adapted to contain test-related material which matches the stressor task. Further, research from our laboratory has demonstrated that the training is successful in modifying emotional responses to the imitation cognitive ability tasks (Mackintosh et al., in prep). This study therefore aims to advance Mackintosh et al.'s study by exploring physiological responses to the paradigm.

As the stressor task has been standardised elsewhere (Hoppitt et al., under review), the present study will not include a control task; all participants will complete the same version of the task. Prior to this, participants will receive CBM-I training that relates specifically to test/examination anxiety. Training will either encourage participants to interpret test-related ambiguous scenarios in a positive manner (positive training) or will draw on the positive and negative interpretations of the scenarios equally (sham training). It is hypothesised, firstly, that participants will find the task emotionally stressful, which will be evidenced by a significant rise in levels of reported stress and negative affect, as well as significant decreases in positive affect. As no main effect of the task on physiological activation was identified in Hoppitt et al., cortisol concentration and sAA output are not predicted to change in response to the task here. However, the direction of CBM-I training is predicted to influence the

magnitude of participants' responses to the task, with positive training leading to a smaller response than sham training. This effect is expected to be evident in both psychological and physiological variables.

7.1 Method

7.1.1 Design

A 2 (between subjects; group: positive training, sham training) x 5 (within subjects; time of saliva samples and mood measurement: baseline 1 and 2, post-stressor, and 20 and 30 minutes post-stressor) mixed model design was used (see Figure 14). Dependent variables, cortisol concentration, sAA secretion, reported stress, and positive and negative affect, were measured at five time points. Dependent variables, state anxiety, reported optimism, happiness, tension, and distress, were measured before and after the stressor. Measures of chronic stress and distress, test anxiety, and trait anxiety were also taken to assess potential influences on participants' vulnerability to stress. Interpretive bias was assessed following CBM-I training (sham or positive) to assess impact of training. Stress was induced through a pseudo cognitive ability test.

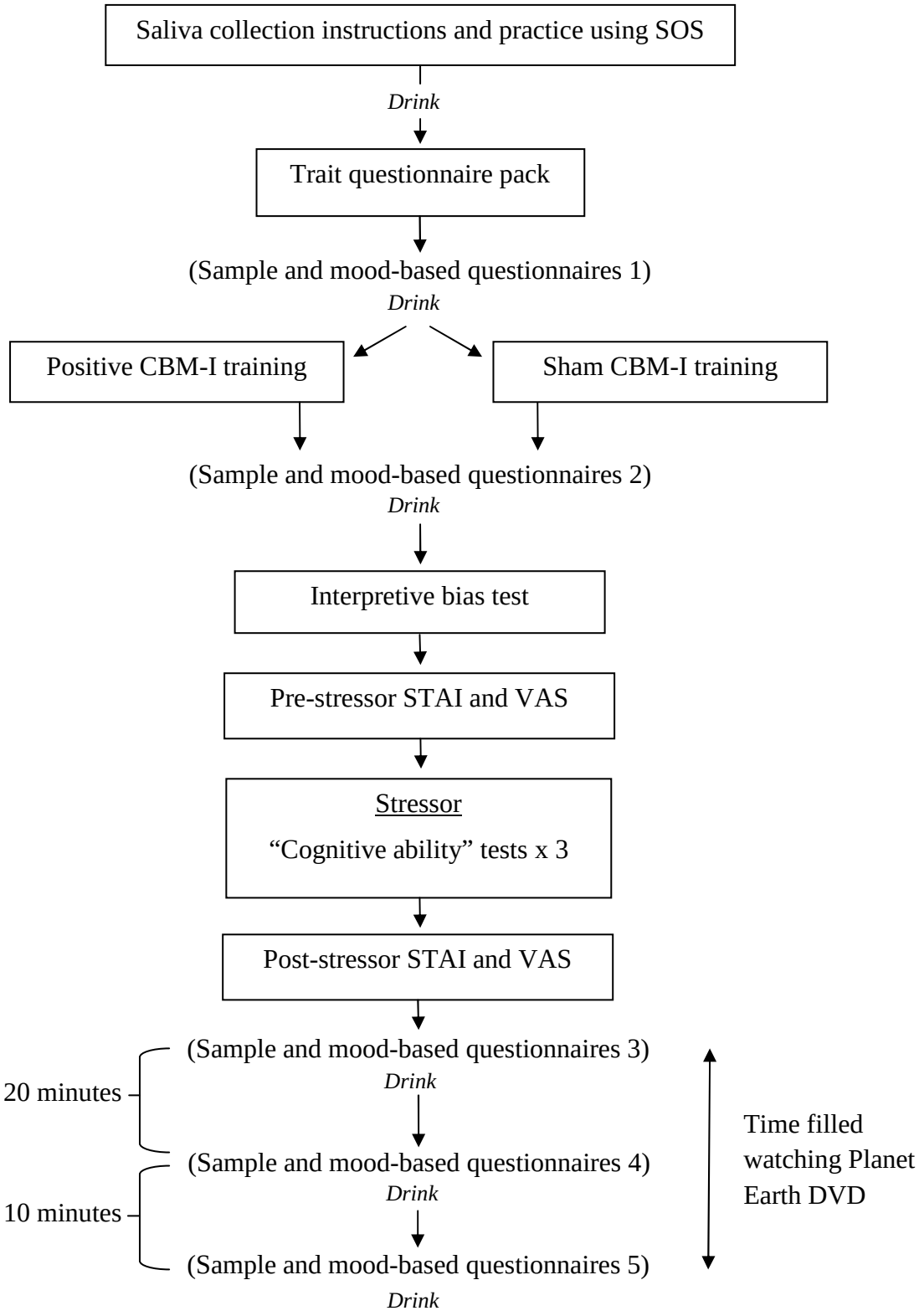

Figure 14. Overview of Study 5's experimental design

7.1.2 Participants

Participants were female students from Anglia Ruskin University, Cambridge, who were aged between 18 and 45 (M = 21.14, SD = 5.11) and reported having English as their first or chosen language. Participants were recruited via an advertisement email, posters displayed around the campus, or from the researcher entering lectures to verbally advertise the study. Students were invited to contact the researcher via email to complete a screening questionnaire (STAI-trait; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). One hundred and twenty six students who scored below 60 on the STAI-trait[8] were invited to participate in the study, of which 83 accepted (group STAI-trait M = 37.16, SD = 9.57). Owing to recruitment techniques, 92.77% of the participant population were from the Faculty of Science and Technology. Of that majority, 68.83% participants studied psychology as either a single or combined honours pathway (55.84% single, 12.99% combined), of which 62.26% were first years, 28.30% were second years, 7.55% were third years, and 1.89% unspecified.

7.1.3 Materials

Questionnaires. Participants completed the GHQ-28, PSS-10, and STAI-trait (as outlined in Study 1). Participants additionally completed the Test-Anxiety Inventory (TAI; Spielberger, 1980), which is a 20-item questionnaire designed to quantify vulnerability to situation-specific anxiety as well as proneness to become emotional and worry in response to taking a test. Participants are required to rate each item according to how they would generally relate to such a situation on a four-point Likert scale ranging from 1 (*Almost never*), to 4 (*Almost always*). Items vary according to whether they refer to anxiety experienced prior to, during, or after an examination. An example of an item on the TAI is *"Even when I'm*

[8] Specified as an ethical requirement.

well prepared for a test, I feel very nervous about it". Responses to items are reversed where necessary and then summed to give an overall measure of test anxiety out of a possible 80 (minimal score of 20). Separate scores for worry and emotionality can also be calculated. Scores from the TAI have been shown to correlate well with alternate measures of test anxiety, such as the Test Anxiety Scale (TAS; Sarason, 1978) (Spielberger, 1980), and the scale also demonstrates good internal consistency (α = .92 - .96; Spielberger, 1980).

Each time participants gave a saliva sample, they completed the SACL and PANAS (details of which can be found in Studies 1 and 2 respectively). Participants additionally completed the STAI-state and VAS measuring optimism, happiness, distress, and tension (see Study 1 for more details on these scales) prior to and immediately after completing the pseudo intelligence test (stressor).

Saliva collection. Participants were issued with instructions on how to give a saliva sample with the use of Salimetrics Oral Swabs (SOS); to passively hold the swab under their tongue for 2 minutes without chewing or sucking it. Excluding the practice sample, participants gave five samples during the study: as an initial baseline approximately 25-30 minutes into the study, a second baseline approximately 75-85 minutes into the study, immediately after the stressor, and 20 and 30 minutes after the stressor. Two baseline samples were taken in consideration of the substantial period of time that had elapsed between the start of the study and the stressor (approximately 120-125 minutes). The third sample was aimed at capturing any immediate sAA response, whilst samples 4 and 5 were aimed at capturing recovered sAA levels and initial cortisol response. Samples were stored in locked freezers at -80°C following the session until needed for analysis, and were analysed for levels of sAA and cortisol. Further details of the assaying procedure can be found in Chapter two.

Interpretive bias training and testing. ***Interpretive bias training (CBM-I).*** A computerised training programme was delivered with the assistance of E-Prime software (Schneider, Eschman, & Zuccolotto, 2002). After receiving instructions and having a chance to practice, participants were presented with 70 scenarios that were presented in seven blocks separated by short breaks. Each scenario was presented one sentence at a time (with no title), and depicted a situation that remained ambiguous as to whether it was positive or negative in nature until the final word which was presented as a word fragment for the participants to solve. In the positive training condition, each scenario was consistently resolved into a positive situation, for example, *"As you work at each new example in a test you find you are not able to solve them in the time given. You assume that you should be able to do the tasks and the time allowed has therefore been carefully chosen so as to be i-p-ss-b—(impossible)"*. Alternatively, in the sham training condition this contingency was not apparent and scenarios resolved into a positive and negative situation (e.g. the negative ending of the previous scenario would end with the word fragment *"e-ou-h (enough)"*) with equal frequency. After each scenario, participants were required to answer a simple comprehension question to further impress the positive, negative, or neutral element to the scenario. For example, the comprehension question relating to the above scenario would be *"Do you think you should finish in the time?"* with the correct answer (yes or no) corresponding to the prior resolved meaning (i.e. positive of negative) of the situation. During each of the seven blocks participants were additionally presented with two filler scenarios that depicted a neutral situation, e.g. *"You attend a schooldays reunion at your old college and meet up with lots of people you have not seen for some time. You speak to lots of old friends and then decide to get a drink. You go to the bar and when you return you find that some of your friends are dancing to loud mu-ic (music)"*.

Bias test. Participants completed an interpretive bias test similar to the one outlined in Study 4. While the participant's task remained the same, for the encoding phase 20 titled scenarios were presented to participants that described situations of being judged either through a test (test anxiety) or by people you would be trying to impress (social anxiety). For example, *"The job interview. You applied for a job in a company you'd really like to work in. You are invited to an interview, where you answer the questions as well as you can. Reflecting later, you think that the quality of your answers decided the ou-com- (outcome)"*. As in Study 4, participants were presented with a simple comprehension question after each scenario, for example *"Did you think about your answers later?"*. Again, after all 20 scenarios had been presented, participants were required to recall each scenario on seeing the title alone and rate four sentences according to their recollection of the initial description. As before, the four sentences included a positive foil (e.g. *"You think it was a good thing you did not take the job"*), a negative foil (e.g. *"You think your poor reference must have made a bad impression"*), a positive target (e.g. *"You think that your astute answers led to you being offered the job"*), and a negative target (e.g. *"You think that your poor answers lost you the job"*) interpretation.

Stressor. Stress was induced using an existing paradigm that has been developed and tested (Hoppitt, Mackintosh, Randall, & Bristow, under review; Mackintosh, Mathews, Eckstein, & Hoppitt, in prep). The method encompasses three difficult computerised cognitive tasks and employs an anticipatory task evaluation. Participants were informed that they would be completing three different computer tasks that measured cognitive ability or intelligence (named *Intelligence 1*, *Intelligence 2*, and *Intelligence 3*). They were further informed that the tasks were specifically designed so that an undergraduate considered to be average in competence should be able to complete the tasks without too much difficulty and that this had been confirmed by recent piloting of the tasks on undergraduates from a nearby

university (University of Cambridge). Participants were informed that performance scores would be automatically transmitted to a laptop set up at the front of the room, which the researcher would compile into an (non-anonymous) table that would be displayed on a large screen following the task to allow participants to see how they compared against their fellow peers in the room. Participants were also informed that, time allowing, those scoring at the top and bottom of the performance table would be asked to reflect publicly on aspects of the task they found particularly easy or challenging. Participants were given 10 minutes to complete the tasks, with time warnings given at the half way point and when two minutes were remaining.

In actual fact, the tasks did not measure intelligence per se, had not been piloted on University of Cambridge undergraduates, were set to be very difficult, and no scores were automatically transferred. These misleading instructions were given to encourage motivation to a good performance, followed by feelings of failure and socio-evaluative threat. For the first task (Intelligence 1), participants were given two minutes to memorise a series of three digit numbers before being asked to recall them backwards, so 321 would need to be recalled as 123. The second task (Intelligence 2) involved participants being given two minutes to learn a series of 14 statements dictating rules governing fictional creatures, such *"all phrups eat soists"* and *"knanges are phrups"*. Participants were then asked to identify a series of correct phrases out of three options to accurately reflect these rules. For the third and final task, participants were set a series of difficult anagrams to solve, e.g. *"raobtomh (bathroom)"*. For each anagram, participants were given a 30 seconds countdown in the corner of the screen before automatically moving on to the next one. This final task (Intelligence 3) included a total of 51 anagrams, with the intention of preventing any participant from completing the three tasks in the set 10 minutes to further induce feelings of failure.

7.1.4 Procedure

Ethical approval was obtained from the NHS Research Ethics Committee. Study sessions were run in groups of up to 11 participants on Anglia Ruskin University campus. Each session started at 1pm on weekdays and weekends and took three hours to complete. Participants were issued with an honorarium of either £20 or 3 research credits (for psychology students). Participants were asked to refrain from eating, drinking (other than water), and smoking for 30 minutes prior to the study, and to abstain from undertaking vigorous exercise for 90 minutes prior to the start. On entry, participants were given information sheets and verbally briefed on the study prior to signing consent forms. To start, participants were taken through the process of giving a saliva sample, including information on sample tracking, before giving practice sample. After this and each subsequent sample, participants were given a cup of mineral water to rehydrate them and optimise successive sample quality. Participants then completed the trait questionnaire pack (titled *Questionnaire Pack 1*), which included the GHQ-28, TAI, STAI, and PSS-10. The first (non-practice) sample was then given, during which participants completed the SACL and PANAS. Participants then completed the CBM-I training (named *Computer task 1*). Participants completed either a positive or a sham training exercise according to their participant number which was assigned on entry to the study on a first come first served basis. All even participant numbers received positive training, while all odd participant numbers received sham training. Participants were given 45 minutes to complete this task, after which the researcher moved the group on to give a second sample and second set of SACL and PANAS scales. After this, participants completed an interpretive bias test (labelled *Computer task 2*). Participants then completed the STAI-state and VAS prior to receiving instructions for and completing the 'Cognitive ability' tasks (CATs). Following the 10 minute limit for the CATs, participants were instructed to stop and switch off their computer screens before completing a

second set of STAI-state and VAS. Participants then gave their third sample and completed the SACL and PANAS. At this point the researcher informed the group that there had been a technical error meaning that not all data had been transferred successfully therefore, in consideration of fairness, the performance evaluation stage would be skipped. While waiting for time to elapse before the final two samples, participants sat and watched a Planet Earth DVD (seasonal forests). After the fifth sample, participants were verbally debriefed and given a written summary of the debrief form, both of which revealed all deception and detailed the aims of the study. After this, participants were asked to sign a re-consent form, in acknowledgement of the masked elements to the study when they initially gave consent. Participants were finally thanked and recompensed for the study.

7.1.5 Data Analysis Plan

Data was explored to ensure it met the assumptions of parametric testing. Participant characteristics were compared between groups to monitor any potential confounds. To test the study hypotheses, repeated measures ANOVAs were conducted on the relevant dependant variables (e.g. reported stress, cortisol concentration, etc) with time as a within-subjects factor. In line with the findings from Hoppitt, Mackintosh, Randall, & Bristow (under review), the influence of test anxiety was included in all analyses that tested the effects of the stressor and the influence of CBM-I. Owing to a small subsample of participants who underwent the stressful task (N = 20), test anxiety was originally considered only as a covariate in Hoppitt et al.. By recruiting more participants and using a within-subjects design in terms of the stressor task, the present study was able to subject the data to a median split to produce a relative high and low test anxiety sample. This post-hoc split was entered into the ANOVA as an independent variable with condition (positive or sham training) as the other independent variable. Main effects of time are reported though not necessarily explored where they were qualified by time x condition interactions. For ease of clarity, main effects

of group or test anxiety, time x test anxiety and group x test anxiety interactions, and three-way (time x group x test anxiety) are largely not reported unless significant or relevant to the point of note. Where appropriate, a priori and post-hoc testing was conducted using paired *t*-tests with Bonferroni corrected alpha levels.

7.2 Results

7.2.1 Data Exploration

Ten participants' data were removed from all analysis due to the participants not completing the CBM-I training within the set 45-minute period. One additional participant's data was removed owing to them rushing through the computer tasks and questionnaires. Of the 72 sets of data included in the analysis, 36 participants were in the positive training condition and 36 participants were in the sham training condition.

Cortisol concentration and sAA secretion data were subjected to log transformation to successfully normalise the distribution of the data. All analyses were conducted using logged data, however graphical representation of the means and measures of variation are presented using unlogged data.

7.2.2 Participant Characteristics

Separate univariate ANOVAs revealed no significant difference between participants in the positive and sham training conditions with regards to their trait questionnaire measures (see Table 18).

Table 18

Mean data for participant trait measures

Measure	Scale	Positive training		Sham training		*F* value	*p* value
		Mean	SD	Mean	SD		
Test anxiety	TAI	38.56	13.22	42.89	14.28	1.79	.19
Trait anxiety	STAI	37.31	9.47	37.24	10.21	.001	.97
Distress	GHQ-28	46.44	8.75	45.51	10.64	.16	.69
Perceived stress	PSS-10	16.23	5.99	15.37	6.44	.33	.57

7.2.3 Interpretive Bias

Data from the CBM-I training was not analysed other than for accuracy. Participants ranged from 70.24 – 96.43% in their overall ability to correctly answer comprehension questions (M = 85.37%, SD = 6.08). A univariate ANOVA identified a significant main effect of condition allocation on accuracy of comprehension questions, $F(1, 70) = 4.01$, $p < .05$, $\eta_p^2 = .05$, with participants allocated to positive training scoring significantly higher (M = 86.77%, SD = 6.30) relative to participants in the sham training condition (M = 83.96%, SD = 5.59).

An interpretive bias index (IBI) score was calculated from the interpretive bias test data in the same manner as discussed in Study 4. First, paired *t*-tests were conducted to distinguish whether participants successfully discriminated between target and foil sentences during the recognition task. Results revealed that participants consistently rated target positive items (M = 2.87, SD = .38) significantly higher with regards to their recollection of

how the sentence matched the original scenario relative to positive foil items (M = 1.85, SD = .34), $t(71) = -19.85$, $p < .001$, $d = 2.83$. Participants similarly rated negative target items (M = 2.25, SD = .39) significantly higher in comparison to negative foil items (M = 1.49, SD = .37), $t(71) = -19.83$, $p < .001$, $d = 1.99$. Negative target ratings of sentences were then subtracted from positive target ratings of sentences to produce an overall IBI score. A higher score indicated a more positive interpretive bias, with lower scores signifying a stronger negative interpretive bias.

A univariate ANOVA was conducted on IBI scores, using condition as a within-subjects variable, to determine whether training had been successful. As hypothesised, IBI scores were significantly higher in the positive training group (M = .77, SD = .59) relative to the sham training group (M = .48, SD = .46), $F(1, 70) = 5.38$, $p = .02$, $\eta_p^2 = .07$. As interpretive bias was measured after the CBM-I training, this result is taken to indicate that training had been successful in improving interpretive bias (positive training) while not affecting interpretive bias (sham training).

7.2.4 Psychological Response to Stressor and CBM-I

State anxiety. A 2 (condition: positive training, sham training) x 2 (test anxiety split: high, low) x 2 (time of measurement: pre-stress, post-stress) repeated measures ANOVA revealed a significant main effect of time, $F(1, 49) = 93.73$, $p < .001$, $\eta_p^2 = .66$. State anxiety was found to significantly increase from an average of 36.28 (SD = 8.84) to 47.60 (SD = 10.37). There was also a significant main effect of test anxiety, $F(1, 49) = 8.44$, $p = .01$, $\eta_p^2 = .15$, with high test-anxious individuals reporting significantly higher levels of state anxiety (M = 45.52, SD = 9.28) relative to low text-anxious individuals (M = 38.83, SD = 8.15). There was no significant main effect of group, $F(1, 49) = 1.34$, $p = .25$, $\eta_p^2 = .03$, and no interactions were found to be significant (all p values > .12).

Reported stress. A 2 (condition) x 2 (test anxiety split) x 5 (time points: baseline, post-CBM, post-stressor, stressor + 20 minutes, and stressor + 30 minutes) repeated measures ANOVA showed a significant effect of time on stress (as measured through the SACL), $F(3.04, 200.67) = 44.20$, $p < .001$, $\eta_p^2 = .40$ (Greenhouse-Geisser). Paired *t*-tests were used to investigate the significant time main effect (see Figure 15). There was no change in reported stress between time points 1-2, $t(71) = -.10$, $p = .92$, $d = .01$. A significant increase in stress was found between time points 2-3, $t(71) = -8.94$, $p < .001$, $d = 1.16$, followed by a significant decrease between time points 3-4, $t(71) = 9.45$, $p < .001$, $d = 1.16$. A further trend decrease in reported stress emerged between time points 4-5, $t(71) = 2.02$, $p = .05$, $d = .17$, from an average of 2.61 ($SD = 3.63$) to 2.04 ($SD = 3.06$).

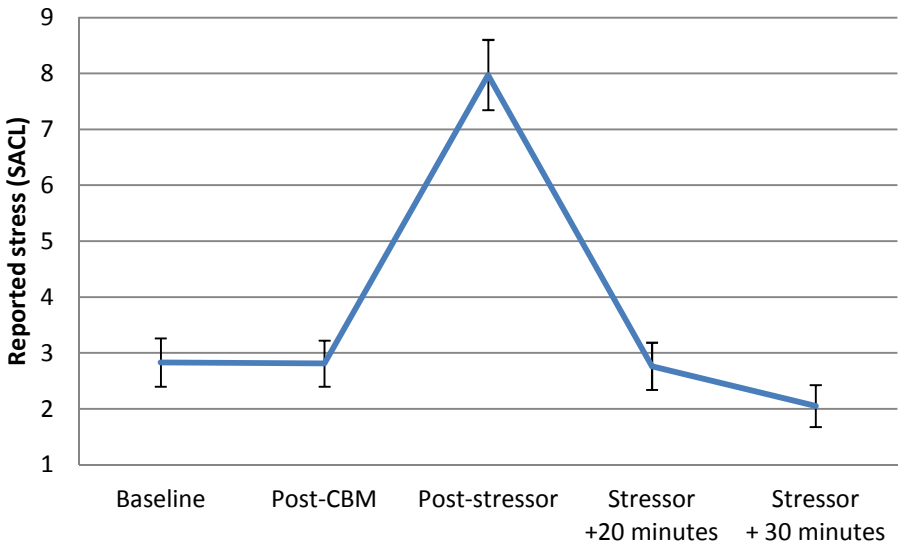

Figure 15. Mean (and SE) reported stress throughout the study (collapsed across conditions)

A significant main effect of test anxiety was also revealed, $F(1, 66) = 44.20$, $p < .001$, $\eta_p^2 = .40$, with high test anxious individuals reporting significantly more stress ($M = 5.03$, $SD = 4.16$) than low test anxious individuals ($M = 2.34$, $SD = 2.75$). There was also a trend main effect of group, $F(1, 66) = 3.66$, $p = .06$, $\eta_p^2 = .05$, with participants who completed positive

training reporting more stress overall (M = 4.32, SD = 3.94) than participants who completed sham training (M = 3.06, SD = 2.97).

There was no significant time x condition interaction, $F(3.04, 200.67) = .38$, $p = .77$, $\eta_p^2 = .01$, and no significant three-way interaction, $F(3.04, 200.67) = .26$, $p = .86$, $\eta_p^2 = .004$. A trend time x test anxiety split interaction emerged, $F(3.04, 200.67) = 2.31$, $p = .08$, $\eta_p^2 = .03$. Exploration of this time x test anxiety trend was carried out by running repeated measures ANOVAs on data from high and low test anxious participants separately. For both high test-anxious (H-TA), $F(3.28, 111.47) = 28.20$, $p < .001$, $\eta_p^2 = .45$, and low test-anxious (L-TA) individuals, $F(2.20, 74.81) = 20.19$, $p < .001$, $\eta_p^2 = .37$, a significant main effect of time was identified. Paired t-tests showed no significant change in reported stress between time points 1-2 for either high, $t(34) = .48$, $p = .64$, $d = .09$, or low, $t(34) = -.92$, $p = .37$, $d = .14$, test anxious individuals. A significant increase in reported stress was found between time points 2-3 for both sub samples, H-TA: $t(34) = -7.48$, $p < .001$, $d = 1.44$, L-TA: $t(34) = -5.04$, $p < .001$, $d = .95$, followed by a significant decrease between samples 3-4, H-TA: $t(34) = 7.36$, $p < .001$, $d = 1.29$, L-TA: $t(34) = 5.65$, $p < .001$, $d = 1.12$. Participants low in test anxiety showed no difference in reported stress from time points 4-5, $t(34) = .36$, $p = .72$, $d = .04$, while high test anxious participants showed a trend decrease, $t(34) = 2.09$, $p = .04$, $d = .28$ (see Figure 16). From Figure 16, and considering the significant main effect of test anxiety, it seems that the trend interaction between time and test anxiety split emerged from high test anxious individuals appearing to be slightly more responsive to the stressor relative to low test anxious individuals. To support this claim statistically, univariate ANOVAs were conducted with percentage change scores as the dependent variable and test anxiety split as the between subjects variable. There was no main effect of test anxiety on stress change scores between measures 1-2, 3-4, or 4-5 (all F values < 1). A trend main effect of test anxiety was found for stress change between measures 2-3, $F(1, 47) = 3.01$, $p = .09$, $\eta_p^2 =$

.06, with H-TA participants reporting a greater change (*M* = 3.69%, *SD* = 4.40) relative to L-TA participants (*M* = 1.81, *SD* = 2.64).

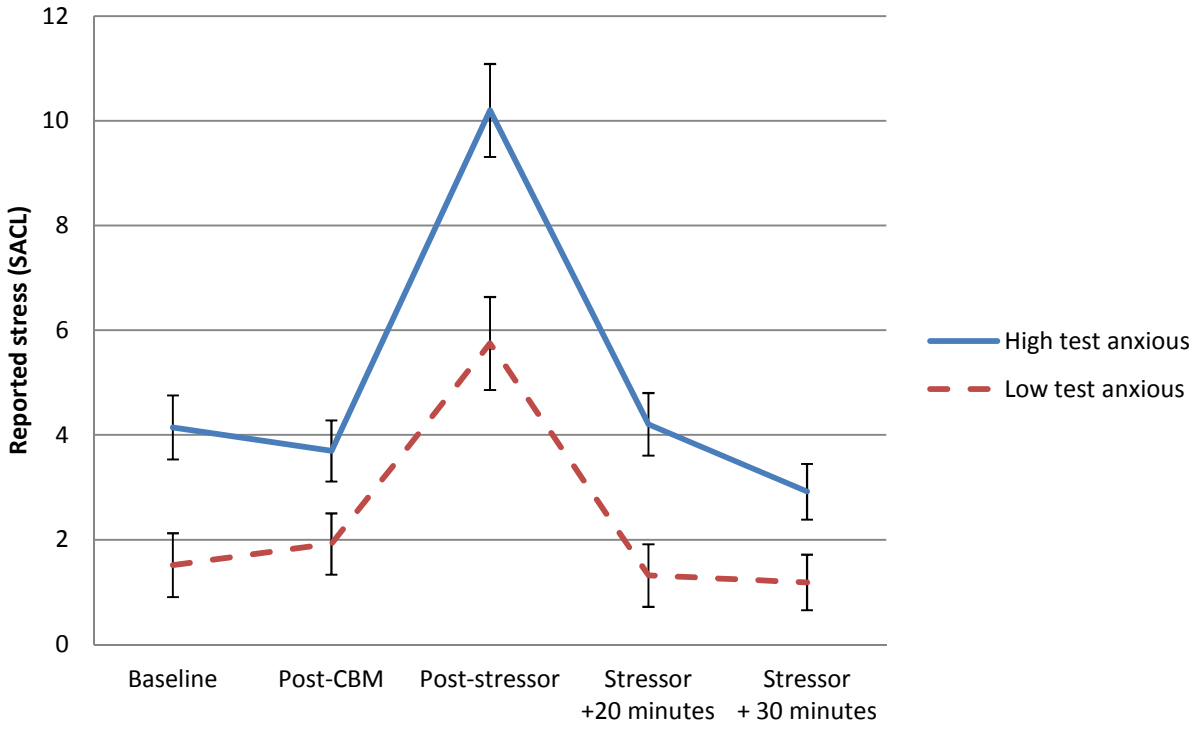

Figure 16. Mean (and SE) reported stress throughout the study according to test anxiety score

Positive affect. A 2 (condition) x 2 (test anxiety split) x 5 (time points) repeated measures ANOVA revealed a significant main effect of time on positive affect as measured through the PANAS, $F(2.84, 184.32) = 12.68$, $p < .001$, $\eta_p^2 = .16$ (Greenhouse-Geisser). Paired *t*-tests were used to investigate the significant main effect. A significant decrease in positive affect was found between time points 1-2, $t(71) = 6.12$, $p < .001$, $d = .52$, from an average of 23.68 (*SD* = 6.87) to 20.24 (*SD* = 6.49). No significant change in positive affect was found between time points 2-3, 3-4, or 4-5 (all *p* values > .09). No other main effects or interactions were found to be significant (all *p* values > .15)

Negative affect. A 2 (condition) x 2 (test anxiety split) x 5 (time points) repeated measures ANOVA revealed a significant main effect of time on negative affect as measured

through the PANAS, $F(2.59, 168.59) = 20.95$, $p < .001$, $\eta_p^2 = .24$. Paired *t*-tests investigated the significant main effect of time (see Figure 17), revealing no change between time points 1-2, $t(71) = -.84$, $p = .40$, $d = .11$. A significant rise in negative affect was identified between time points 2-3, $t(70) = -5.08$, $p < .001$, $d = .64$, followed by a significant decrease between time points 3-4, $t(70) = 7.01$, $p < .001$, $d = .65$, and 4-5, $t(71) = 2.67$, $p = .01$, $d = .15$.

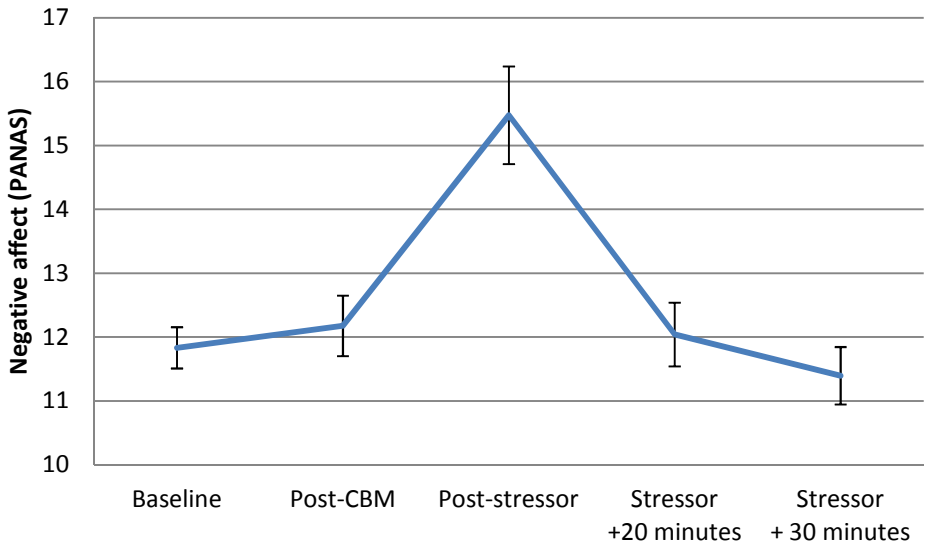

Figure 17. Mean (and SE) negative affect throughout the study (collapsed across conditions)

A significant main effect of test anxiety was also revealed, $F(1, 65) = 8.40$, $p = .01$, $\eta_p^2 = .11$, with H-TA participants reporting significantly more negative affect ($M = 13.78$, $SD = 4.73$) relative to L-TA individuals ($M = 11.39$, $SD = 2.49$). Further, a trend time x test anxiety interaction was found, $F(2.59, 168.59) = 2.31$, $p = .09$, $\eta_p^2 = .03$.[9] No other main effects or interactions were significant (all *p* values > .16).

Psychological response summary. State anxiety, reported stress and negative affect all respond as hypothesised to the stressor, showing an acute increase. Reported stress and negative affect appear to recover quickly from the response. Following a decrease after completing CBM, there is no change in positive affect throughout the study. Participants who

[9] Upon further investigation, this trend showed the same patterns as was found in the stress data. This trend is suggested to be caused by the significant main effect of group, hence further investigations are not reported.

have high test anxiety reported significantly more anxiety, stress, and negative affect overall. CBM-I training appeared to exert no influence to emotional responses to the stressor task.

7.2.5 Physiological Response to Stressor and CBM-I

Cortisol concentration. A 2 (condition: positive, sham training) x 2 (test anxiety split: high, low) x 5 (time points: baseline, post-CBM, post-stressor, stressor + 20 minutes, stressor + 30 minutes) repeated measures ANOVA was conducted with cortisol concentration as the dependent variable. A significant time main effect was revealed (see Table 19), $F(2.21, 121.75) = 59.65$, $p < .001$, $\eta_p^2 = .52$ (Greenhouse Geisser). Paired *t*-tests revealed a significant decrease in cortisol between samples 1-2, $t(64) = 10.75$, $p < .001$, $d = .66$, and 2-3, $t(66) = 3.50$, $p = .001$, $d = .26$, and a trend decrease between samples 4-5, $t(63) = 1.99$, $p = .05$, $d = .11$. No change was observed between samples 3-4, $t(64) = .11$, $p = .92$, $d = .01$.

Table 19

Mean (µg/dL) and variance of cortisol concentration throughout the study

	Baseline	Post-CBM	Post-stressor	Stressor + 20 minutes	Stressor + 30 minutes
Mean	.22	.15	.12	.12	.12
SD	.19	.09	.06	.06	.06

No significant main effect of test anxiety was identified, $F(1, 55) = .34$, $p = .56$, $\eta_p^2 = .01$, though a trend condition main effect emerged, $F(1, 55) = 3.01$, $p = .09$, $\eta_p^2 = .05$, with participants in the sham training group showing slightly higher levels of overall cortisol ($M = .15$µg/dL, $SD = .07$) compared with participants in the positive training group ($M = .14$µg/dL, $SD = .11$). No significant time x condition interaction, $F(2.21, 121.75) = .37$, $p = .71$, $\eta_p^2 =$

.01, or time x test anxiety split interaction, $F(2.21, 121.75) = 1.81$, $p = .16$, $\eta_p^2 = .03$, was identified, though a significant three-way time x condition x test anxiety interaction emerged, $F(2.21, 121.75) = 3.21$, $p = .04$, $\eta_p^2 = .06$ (see Figure 18).

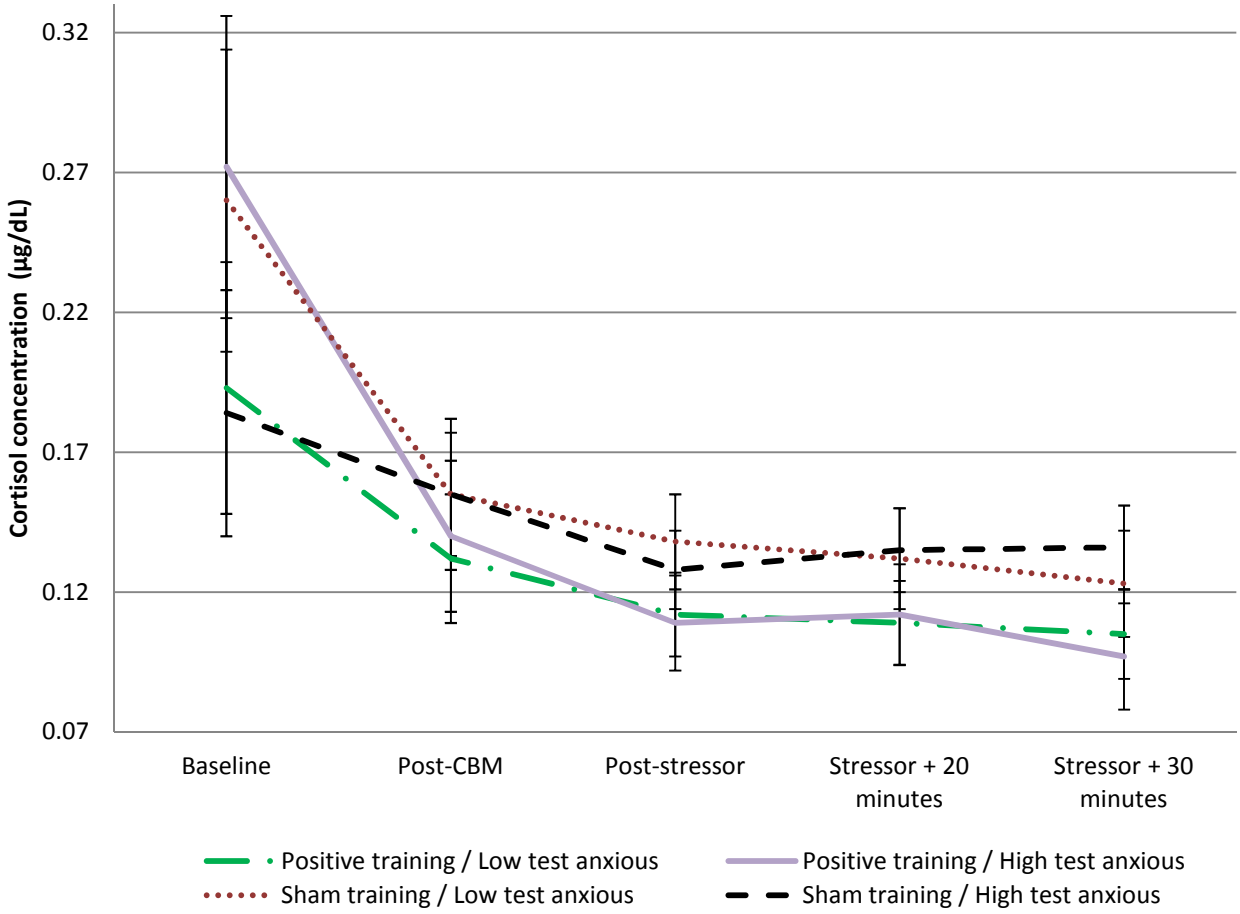

Figure 18. Demonstrating the significant three-way interaction (time x condition x test anxiety) for cortisol concentration.

To explore the three-way interaction, a series of 2 (condition) x 2 (test anxiety split) x 2 (time points) repeated measures ANOVAs were conducted with cortisol data comparing time points 1-2, 2-3, 3-4, and 4-5 (separately) as the dependent variable (see Table 19 or Figure 18 for a reminder of time points). A significant main effect of time was identified between time points 1-2 (baseline – post-CBM), $F(1, 59) = 151.79$, $p < .001$, $\eta_p^2 = .72$, which

was qualified by a significant three-way time x condition x test anxiety interaction, $F(1, 59) = 12.79$, $p = .001$, $\eta_p^2 = .18$.

To explore the three-way interaction separate repeated measures ANOVAs were conducted on high and low test anxious participants' data individually, using condition (sham training, positive training) as a between subjects factor and time point (1-2) as a within subjects factor. For low test anxious individuals, there was a significant main effect of time on cortisol concentration, $F(1, 28) = 93.74$, $p < .001$, $\eta_p^2 = .77$, which was qualified by a trend time x condition interaction, $F(1, 28) = 3.05$, $p = .09$, $\eta_p^2 = .10$ (see Figure 19). Paired *t*-tests showed a significant decrease in low test anxious participants who experienced both positive, $t(17) = 7.38$, $p < .001$, $d = .60$, and sham training, $t(11) = 6.17$, $p < .001$, $d = 1.64$, though Figure 19 seems to show a marginally steeper decrease for low test anxious participants who receive sham training.

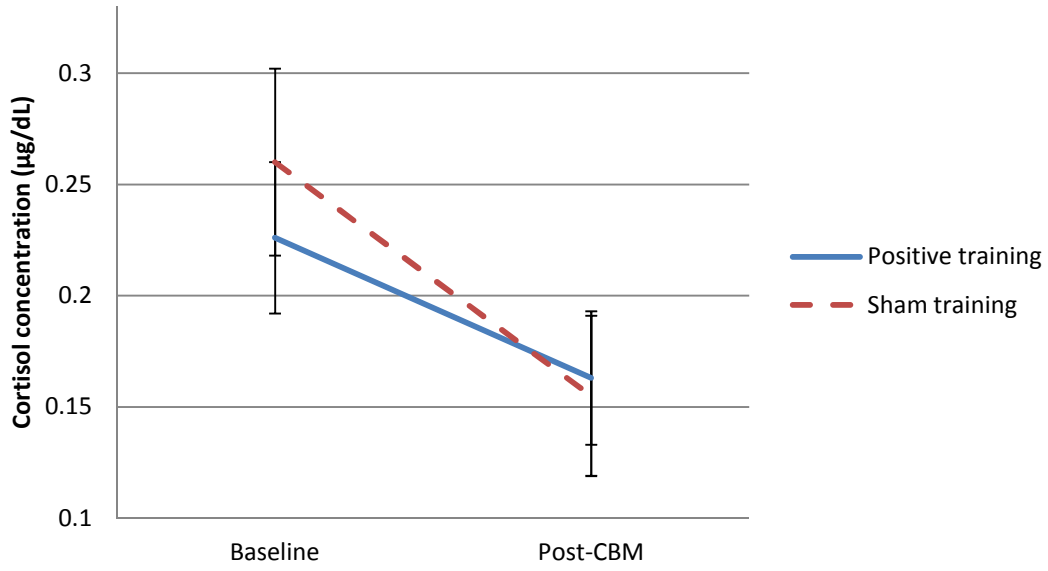

Figure 19. Mean change (and standard error) in cortisol concentration between time points 1-2 for low test anxious participants.

For high test anxious participants, a significant main effect of time on cortisol concentration was found between time points 1-2, $F(1, 31) = 61.00$, $p < .001$, $\eta_p^2 = .66$, which was qualified by a significant time x condition, $F(1, 31) = 10.90$, $p = .002$, $\eta_p^2 = .26$. Paired *t*-tests revealed a significant decrease in cortisol for high test anxious individuals who received either positive, $t(11) = 7.77$, $p < .001$, $d = .66$, or sham training, $t(20) = 3.55$, $p = .002$, $d = .45$, though Figure 20 suggests a steeper decrease in cortisol in high test anxious individuals who received positive training.

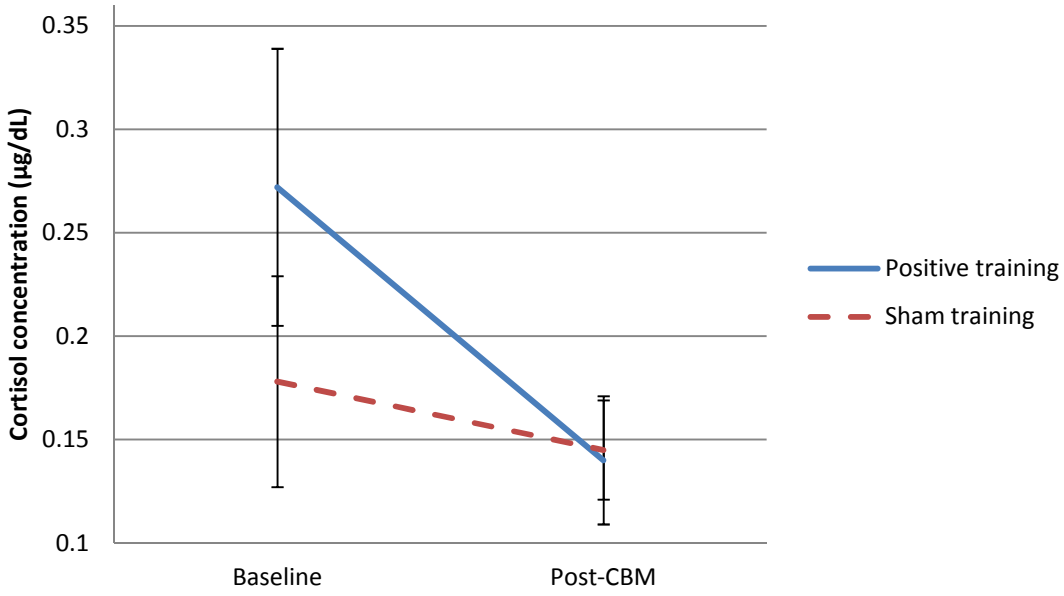

Figure 20. Mean change (and standard error) in cortisol concentration between time points 1-2 for high test anxious participants

A significant main effect of time was identified between time points 2-3 (post-CBM – post-stressor), $F(1, 61) = 8.66$, $p = .01$, $\eta_p^2 = .12$, evidencing a decrease in cortisol from a mean of .15µg/dL (*SD* = .11) to .13µg/dL (*SD* = .07). However, no significant main effect of time was found between time points 3-4 (post-stressor – stressor + 20 minutes), $F(1, 59) = .02$, $p = .90$, $\eta_p^2 < .001$. No further significant main effects or interactions were found between either of these time point comparisons (all *p* values > .15).

A significant main effect of time was found between time points 4-5 (stressor + 20 minutes – stressor + 30 minutes), $F(1, 58) = 5.77$, $p = .02$, $\eta_p^2 = .09$, which was qualified by a significant three-way time x condition x test anxiety interaction, $F(1, 58) = 5.85$, $p = .02$, $\eta_p^2 = .09$. To explore this three-way interaction separate repeated measures ANOVA were conducted on high or low test anxious participants' data, using condition as a between-subjects factor and time as a within-subjects factor.

For low test anxious participants, there was no significant main effect of time, $F(1, 29) = 2.65$, $p = .12$, $\eta_p^2 = .08$, or condition, $F(1, 29) = .80$, $p = .38$, $\eta_p^2 = .03$, nor any significant time x condition interaction, $F(1, 29) = 2.37$, $p = .14$, $\eta_p^2 = .08$. For high test anxious participants, a trend main effect of time on cortisol concentration was identified between time points 4-5, $F(1, 29) = 3.18$, $p = .09$, $\eta_p^2 = .10$, which was further qualified by a trend time x condition interaction, $F(1, 29) = 3.49$, $p = .07$, $\eta_p^2 = .11$. Post-hoc investigations using paired *t*-tests support the visual interpretation (see Figure 21). High anxious participants who received positive training show a trend decrease in cortisol concentration, $t(11) = 2.15$, $p = .06$, $d = .34$ (Bonferroni corrected $\alpha = .03$), whereas high anxious participants who received sham training show no change in cortisol concentrations between these time points, $t(18) = -.07$, $p = .94$, $d = .01$.

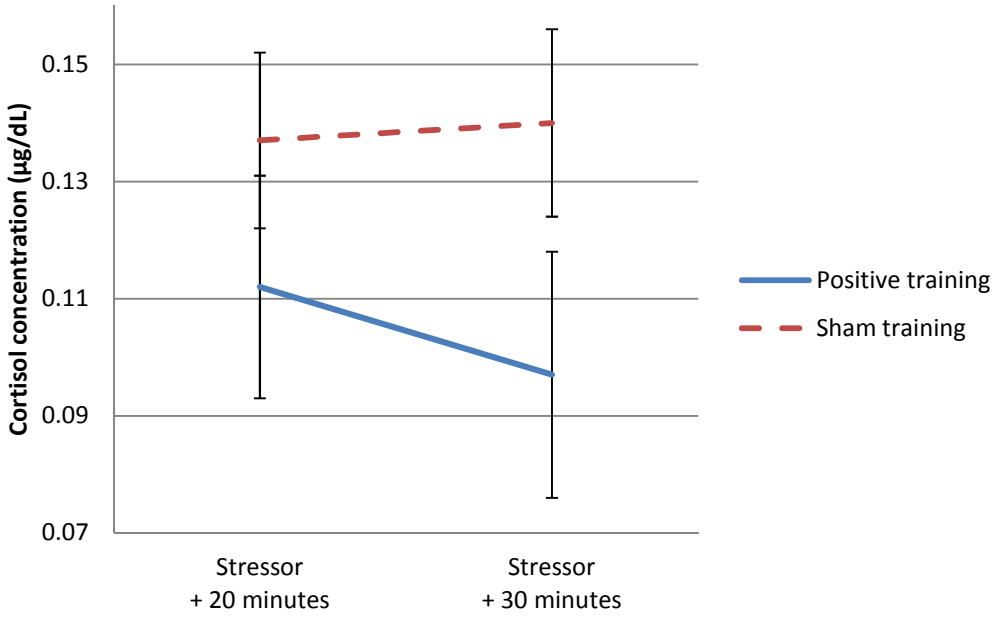

Figure 21. Mean change (and standard error) in cortisol concentration between time points 4-5 for high test anxious participants

Cortisol summary. In support of the hypothesis, findings showed no significant main effect of time in response to the stressor. In contrast to the hypothesis, no time x condition interaction was observed, implying that CBM-I training had no effect on cortisol response to the stressor. However, some interesting patterns emerge relating to condition assignment and test anxiety. Participants high in test anxiety differed in their cortisol response between the last two samples during the recovery phase, with the positive training group showing a decrease in cortisol while the sham training group showed no change in cortisol.

Alpha amylase secretion. A 2 (condition: positive, sham training) x 2 (test anxiety split: low, high) x 5 (time points: baseline, post-CBM, post-stressor, and 20 and 30 minutes post-stressor) repeated measures ANOVA was conducted on sAA secretion data. A significant main effect of time was identified, $F(4, 188) = 3.38$, $p = .02$, $\eta_p^2 = .07$ (see Figure 22), which was qualified by a trend three-way time x condition x test anxiety interaction, $F(4, 188) = 2.09$, $p = .08$, $\eta_p^2 = .04$.

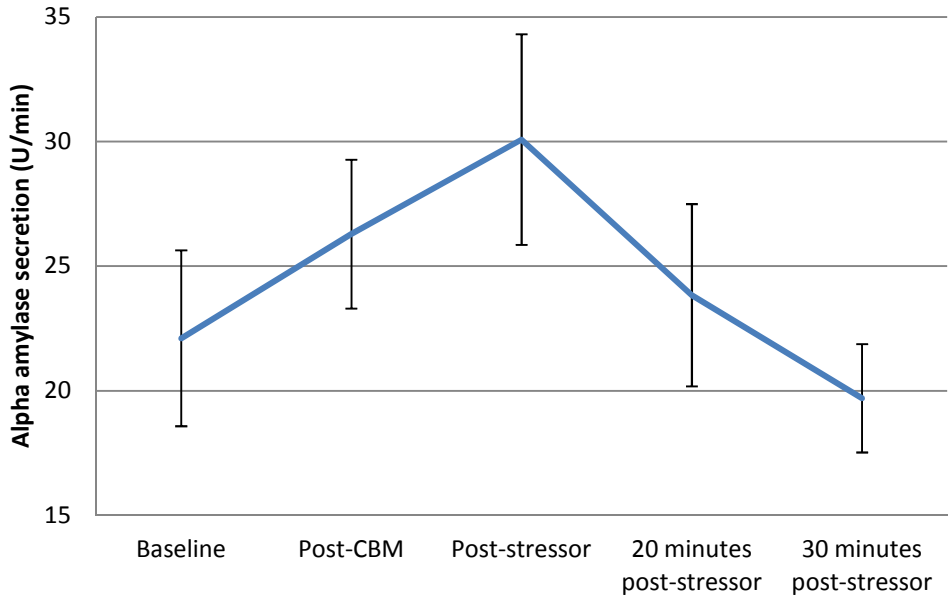

Figure 22. Changes in sAA secretion over the study (collapsed across conditions)

To explore the significant main effect of time and trend three-way interaction, a series of 2 (condition) x 2 (test anxiety split) x 2 (time points) repeated measures ANOVAs were conducted with comparing time points 1-2 (baseline – post-CBM), 2-3 (post-CBM – post-stressor), 3-4 (post-stressor – 20 minutes post-stressor), and 4-5 (20 – 30 minutes post-stressor).

For time points 1-2, a significant main effect of time was found, $F(1, 57) = 5.88$, $p = .02$, $\eta_p^2 = .10$, showing a significant increase in sAA secretion from an average of 18.85U/min (*SD* = 24.57) to 23.54U/min (*SD* = 22.18). No other significant main effects or interactions emerged (all *p* values > .10). No significant main effects or interactions were identified between time points 2-3 (all *p* values > .10).

Between time points 3-4, a main effect of time was identified, $F(1, 54) = 10.00$, $p = .003$, $\eta_p^2 = .16$, showing a significant decrease in secretion from an average of 26.72U/min (*SD* = 29.78) to 21.73U/min (*SD* = 25.59). No other significant main effects or interactions were found (all *p* values > .30). Between time points 4-5, no significant main effects of time,

condition, or test anxiety, nor any significant time x condition/test anxiety or condition x test anxiety interactions were found (all *p* values > .14). However, a significant three-way interaction between time, condition, and test anxiety split was identified, $F(1, 51) = 5.43$, $p = .02$, $\eta_p^2 = .10$. To further explore this, separate repeated measures ANOVAs were run on sAA secretion data from low and high test anxious individuals using condition as a between-subjects factor and time as a within-subjects factor. For high test anxious individuals, no significant main effects or interactions were revealed (all *p* values > .56). For low test anxious individuals, there was no significant main effect of time, $F(1, 26) = .02$, $p = .89$, $\eta_p^2 = .001$, or condition, $F(1, 26) = 1.14$, $p = .30$, $\eta_p^2 = .04$, though a significant time x condition interaction was identified, $F(1, 26) = 8.22$, $p = .01$, $\eta_p^2 = .24$. Paired *t*-tests revealed no significant difference between the two time points for low test anxious individuals who received sham training, $t(10) = 1.51$, $p = .16$, $d = .33$. However a significant increase in sAA secretion was identified between the time points in low test anxious individuals who received positive training, $t(16) = -2.80$, $p = .013$, $d = .34$, from an average of 14.24U/min (*SD* = 16.69) to 16.68U/min (*SD* = 13.78).

Alpha amylase secretion summary. No change in sAA secretion was observed between samples 2-3 following the stressor, which supports the hypothesis. However no time x condition interaction was revealed, which fails to support the hypothesis. These findings suggest that CBM-I training has no effect on sAA response to stress. The secretion rate of sAA significantly increased following CBM-I training and decreased 20 minutes after the stressor. For low anxious individuals who had received positive CBM training, a further significant increase was found between 20 and 30 minutes after the stressor.

7.3 Discussion

The hypothesis that positive CBM-I training would lead to reduced emotional and physiological vulnerability to stress was largely not supported. Condition allocation (sham or positive training) had no influence on psychological or physiological responses to the imitation cognitive ability tasks. However, in line with the predicted response, there was a trend for higher levels of cortisol and significantly greater levels of reported stress overall in participants who received sham training as opposed to positive training.

Though not forming part of the initial hypothesis, the data suggests that the process of completing a single session of CBM-I training influenced psychological state. Positive affect was found to decrease significantly over the 45-minute training period. Cortisol was also found to decrease during this time, while sAA was found to increase, however these physiological patterns are likely due to the natural diurnal variations that would be expected in the afternoon (Kirschbaum & Hellhammer, 1989; Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007). Condition allocation was not shown to interact with the decrease in positive affect, suggesting the changes occurred in response to completing the task rather than training content (i.e. positive or sham). This finding implies that completing CBM-I training led to short-term negative psychological effects. However, no increases in negative affect or stress were observed, suggesting that while positive mood might have been decreased, negative mood was not increased. This is an important finding in terms of participants' willingness to complete such training in a real life setting. In a pilot study, Brosan, Hoppitt, Shelfer, Sillence, and Mackintosh (2011) collected information regarding the acceptability of both CBM-A and CBM-I training procedures. CBM-A was perceived by some as "boring", while CBM-I was seen as more helpful in making participants (who were clinically anxious) more aware of their negative thinking styles. However, participants in Brosan et al.'s study were given prior information alerting them to the fact that the tasks were designed to

(positively) change their thinking styles. Therefore, compliance might possibly have been greater in their sample (N = 12), as completion of the tasks had some implied personal benefit. In the present study, however, participants were not informed that the tasks were designed to modify cognitions, therefore they might have viewed it more similarly to the CBM-A training (i.e. repetitive and without purpose). Taken together, these findings demonstrate that participants appear to require some motivation to complete the tasks, either through payment (present study) or perceived psychological benefits (Brosan et al.'s study), though the momentary effects on mood can be quite different. Supposing CBM training is considered boring, compliance regarding training frequency in a clinical setting is likely to be significantly reduced in people who struggle to maintain motivation as a side-effect of their condition (e.g. people suffering from depression). Therefore future research might look to address these issues by making training sessions shorter or more varied.

The finding that CBM-I training failed to amend psychological responses to an acute laboratory stressor is surprising given the amount of literature that has documented such a response (e.g. Mackintosh, Mathews, Yiend, Ridgeway, & Cook, 2006; Yiend, Mackintosh, & Mathews, 2005). However, it is important to note that the majority of previous studies isolating such an effect have included a negative training condition whereas the current study employed the use of sham training. Consequently, the differences between the two groups might have been somewhat muted compared to two conditions that train in entirely opposite directions. The use of sham training in preference to negative training was justified through ethical considerations. Prior research has confirmed that negative training is successful in training a more negative bias (e.g. Mathews & Mackintosh, 2000). As the enduring effects of even a single session of CBM are being recognised (e.g. Mackintosh et al., 2006), it seems ethically irresponsible to continue using negative training in research. In acknowledgement of the absence of training effects being apparent when comparing positive with sham training,

future research might seek to resolve these ethical concerns in other manners. For example, the use of negative training might be more acceptable assuming efforts are made to extinguish any enduring effects (e.g. subsequent delivery of positive training).

Similar to the findings of Study 4, evidence emerged to suggest that interpretive bias training might be linked more with how participants recover from acute episodes of stress, rather than the extent of their initial response. However, in the current study these patterns were dependent on trait levels of test anxiety. Participants who had high levels of test anxiety and who completed positive CBM-I training were found to show a trend decrease in cortisol between the final two samples. Alternatively, this decrease was absent from high anxious participants in the sham training group and from all low anxious individuals. These findings might be interpreted to suggest that positive training aided recovery from the stressor, but only when participants reported high levels of test anxiety. At the same time, participants with low levels of test anxiety who received positive training showed a significant increase in sAA secretion between these final two samples. While sAA secretion was shown to recover from the stressor between the previous two samples, this finding might still signal some interference in recovery. Taken together, this suggests that high test anxious participants recovered quicker following positive training while low test anxious individuals recovered more successfully following sham training.

To date no study has investigated the physiological effects in response to stress following interpretive bias training. The only published study that has investigated CBM-A and the physiological stress response provides evidence to suggest that positively-trained attentional biases lead to a reduced physiological reaction to stress (Dandeneau, Baldwin, Baccus, Sakellaropoulo, & Pruessner, 2007). This ties in with the few published studies that have looked at natural attentional biases and the physiological stress response (e.g. Fox, Cahill, & Zougkou, 2010), who have also documented influences in terms of initial response.

However, while evidence from Study 4 and that presented here implicate interpretive biases in the recovery stage following stress, it is not possible to state that attentional bias does not additionally influence in this stage. Study 4 failed to find evidence of any influences of attentional bias, however Fox et al. only monitored the initial response stage to stress without including a recovery/follow-up measure. Further, it is possible to interpret Dandeneau et al.'s results as evidence either for a reduced response or a quicker recovery (or both). Dandeneau et al. took measurements of cortisol throughout the working day to assess any shift in general levels of work stress. Their finding of reduced overall cortisol following CBM-A training might therefore indicate either a reduced initial response to stressors or an improved recovery. Consequently, either explanation would result in the observed overall lower levels of cortisol. In terms of the method's clinical potential, both helping to reduce the initial propensity to engage with negative stimuli and encouraging effective recovery from instances of stress should logically produce beneficial outcomes. Nevertheless, further research should aim to provide a clearer understanding of the areas of influence in which bias training might be effective.

An obvious limitation to the current study is the absence of any measure of interpretive bias prior to the training. This was an intentional omission in light of the already lengthy time commitment required from participants. A univariate ANOVA on IBI scores showed that, following CBM-I training, participants in the positive training condition had a significantly more positive interpretive bias compared to sham-trained participants. This was taken to indicate that training had been successful, especially considering the finding of no significant differences between conditions in trait measures of general or test specific anxiety. However, it is recognised that this deduction can only ever be supposed and not conclusively drawn without a baseline measure against which to compare. Therefore, while this assumption is still held, a future study aiming to further explore the interpretations drawn

here might be advised to alter the design to incorporate a baseline bias measure. This might reasonably lead to the study being conducted over a number of sessions to avoid fatigue effects.

To summarise, the present study successfully adopted a stressful task to investigate the influences of training an interpretive bias on the psychological and physiological stress response. While no evidence emerged to support previous findings of CBM-I training reducing emotional vulnerability to stress, there was further indication to support previous suggestions that interpretive biases influence how efficiently people recover (on a physiological scale) from stressful events. Further research is necessary to clarify interactions that emerged implicating trait anxiety (specific to the test) in this relationship. Of practical significance, research is also recommended to investigate methods of making the training more enjoyable to optimise the chances of people opting to complete the tasks without obvious forms of compensation.

8.0 CHAPTER EIGHT: STUDY SIX

Testing the immediate robustness of a single session of CBM training

Overview

Findings from Study 4 indicate a tentative link between an individual's cognitive bias and aspects of their physiological response to acute stress. Specifically, interpretive bias was found to predict recovery of sAA secretion following participation in an online simulated live web chat. Furthermore, Study 5 demonstrated the impact of training participants toward a more positive bias on the physiological response to a stressor. Participants who received positive CBM-I training showed trend lower levels of cortisol over the study relative to participants who completed sham training. Further, an interaction between trait test anxiety and training emerged. Participants with higher levels of test anxiety appeared to show improved recovery from a stressful episode following positive CBM-I training. Alternatively, low test anxious participants were argued to recover better following sham training. The findings from these studies do appear to provide tentative evidence supporting the role of cognitive bias in the physiological stress response. However, it is difficult to draw general conclusions relating to the actual utility of CBM procedures in real life based on these findings. For example, little can be said regarding the longevity of the training effects other than to say that an individual's response to a stressor appears to be influenced by training when the stressor is presented immediately after training. A review of the literature suggests that comparatively less attention has been given to researching factors, such as longevity, relative to the amount of time spent exploring the potential of CBM in different populations and situations. Given the apparent importance of cognitive bias in the stress response, this study seeks to conclude the experimental research in this thesis by investigating the ease with which interpretive and attentional biases are induced and their robustness in the face of adversity.

Introduction

In 1986 MacLeod, Mathews, and Tata documented what we now consider to be the characteristic inverse relationship between anxiety and bias, where participants who have a more negative attentional bias (i.e. those who preferentially attend to threatening stimuli over positive stimuli) typically have higher levels of anxiety than participants with a more neutral or positive bias. Other pioneering studies were able to demonstrate that modifying either attentional or interpretive biases had consequential effects on anxiety levels (e.g. Grey & Mathews, 2000; MacLeod, Rutherford, Campbell, Ebsworthy, & Holker, 2002; Mathews, Richards, & Eysenck, 1989). Following these influential initial studies, the focal point of research in this area appears to have rapidly progressed onto more complex explorations, such as investigating the effectiveness of CBM in a range of clinical disorders. For example, we now know that CBM methods are effective in improving negative biases (by making them more positive) in normal, high and clinically anxious samples (e.g. Amir, Weber, Beard, Bomyea, & Taylor, 2008; See, MacLeod, & Bridle, 2009; Schmidt, Richey, Buckner, & Timpano, 2009). Indeed, one study focusing on individuals with Generalised Anxiety Disorder found that 50% of participants who had completed eight sessions of CBM-A over a four week period no longer met the diagnostic criteria for the disorder, relative to 13% of participants in the control condition (Amir, Beard, Burns, & Bomyea, 2009).

Research interests are also increasingly focusing on how modifying biases can change individuals' physiological responses to stress. For example, Dandeneau, Baldwin, Pruessner, Baccus, and Sakellaropoulo (2007) showed how completion of an attentional modification procedure once a day for five days resulted in decreased levels of cortisol in a group of telemarketers, relative to those who completed a control task. Findings from work presented in this thesis (Studies 4 and 5) additionally support the notion of a relationship between both natural and trained biases on how individuals physiologically respond to stressful aspects of

their environment. Both studies, for example, provided evidence to suggest that interpretive biases might influence physiological recovery success following a stressor, which seems consistent with recent research investigating the role of bias training on the physiological stress response (Baert, Casier, & De Raedt, 2011). Such findings are important both for their theoretical and clinical significance, by enabling a better understanding of the effects of natural and manipulated biases which, ultimately, might lead to the development of a clinical tool. It seems, therefore, that the experimental designs and concepts are expanding at an exponential rate in attempts to understand biases and explore the potential of associated training techniques.

In the excitement of exploring the potential of these techniques, certain important considerations relating to the validity and reliability of the methods appear to have either been overlooked or only modestly investigated. For example, the method most commonly used to test an interpretive bias, the recognition test, has only very recently been validated as an appropriate manipulation check (Salemink & van den Hout, 2010a). As another example of a basic yet necessary investigation, Yiend, Mackintosh, and Mathews (2005) only relatively recently demonstrated how the effects of a single training session endure over a 24-hour period. Mackintosh, Mathews, Yiend, Ridgeway, and Cook (2006) extended this by finding that the effects of training were maintained despite changes between training and testing phase contexts (testing room and modality of presentation). This was a critical finding in furthering the technique's clinical potential because it suggests that the effects of CBM could generalise outside the laboratory. Furthermore these researchers showed how, on the second day, preserved training effects were strong enough to influence responses to a stressor task to a level that would be expected had the stressor been exposed immediately after initial CBM training. The effects of multiple sessions of CBM have also been investigated, with

evidence suggesting that following four sessions of training the effects endure for one week (Mathews, Ridgeway, Cook, & Yiend, 2007).

A further important point that appears to have been initially overlooked is whether fluctuations in mood interferes with inferred training effects. It is surprising that this issue has only recently been addressed, given that critics of the field commonly refer to this as a major weakness of the area. Salemink and van den Hout (2010b) explored this question and found interpretive bias modification to be independent to changes in mood. In support of this conclusion, research that starts to identify physiological changes following CBM are also increasingly able to rule out the presence of such demand effects in addition to obtaining a better command of the breadth of the training's promise.

These types of studies, while relatively basic and to some extent logically assumed, remain necessary in order to justify the investment of resources into the development of CBM as a readily accessible clinical tool. The current study aims to focus on a still largely neglected issue; investigating the robustness of a single session of CBM training. This issue is essential in order to assess the durability of training for methodological reasons, such as how best to structure training sessions. Furthermore, the results of this study will also provide insight into the potential longevity of the wide ranging effects of CBM training, such as how long the protective effects (both psychologically and physiologically) might be evident for.

It is currently known that, for individuals suffering from Social Anxiety Disorder, clinical improvements following attentional bias training are maintained at a 4-month follow up (Schmidt et al., 2009). For unselected participants, it is known that the effects of a single session of CBM can last at least 24 hours (Yiend et al., 2005). However, no direct attempts have been made to extinguish the effects of training during the time between training and testing a bias in these studies. It is therefore possible that the effects of training remain

apparent when tested at a later date because there have been few opportunities that challenge the training during this interluding time. This is especially likely for Yiend et al.'s study, due to the relatively short interval (24-hours) between training and testing a bias.

The current study will therefore expose a freshly trained bias to an equal amount of untraining[10] with the aim of determining how impervious newly trained biases are. Study 6a will focus on attentional bias and Study 6b will focus on interpretive bias. For both experiments, participants will complete three bias tests; one for a baseline measure, one immediately after training, and one immediately after 'untraining'. It is hypothesised, firstly, that training will be effective in both experiments, which will be evidenced by a significant increase in bias index scores (attentional or interpretive) from test 1 to test 2 indicating a more positive bias. Secondly, the effects of training are predicted to generalise from training material to new material, which will be evidenced by increases in positive bias index scores in both old and new test stimuli at test 2. Thirdly, from the current literature that shows a persistence of training effects up to 4 months following initial training, it is hypothesised that untraining will be ineffective in extinguishing training effects, which will be evidenced by no change in bias index scores (attentional or interpretive) for either stimuli type (old or new) from test 2 to test 3.

[10] It is acknowledged that this 'untraining' phase has a purpose of testing a freshly trained bias, rather than specifically aiming to extinguish a bias with directed counter training. For ease of expression, 'untraining' has been selected for reference to this stage.

8.1 STUDY 6A

8.1.1 Method

8.1.1.1 Design

This study utilised a repeated measures design, with one independent variable being the time of CBM test (pre-training, post-training, and post-untraining) (see Figure 23). The dependent variable was the participant's reaction time to respond to targets presented behind either negatively valenced or neutral words, which was condensed to a single attentional bias index (ABI) score. To calculate ABI scores, median reaction time (in milliseconds) to respond to probes behind positive words was subtracted from median reaction time to respond to probes placed behind negative words. The resulting index score provided a measure of attentional bias that represented a continuous variable, with a more positive score indicating a more positive bias and vice versa. This method was adapted from Macleod et al. (1986), and is a common technique used in more recent research.

8.1.1.2 Participants

Participants (N = 39; 28 females) consisted of staff and students at the University of East Anglia, who were recruited through bulletin email advertisements, departmental and university-wide website advertisements, and study posters placed across the campus. The sample was aged between 18 and 60 and mean trait anxiety levels of the sample was 44.32 (SD = 10.12).

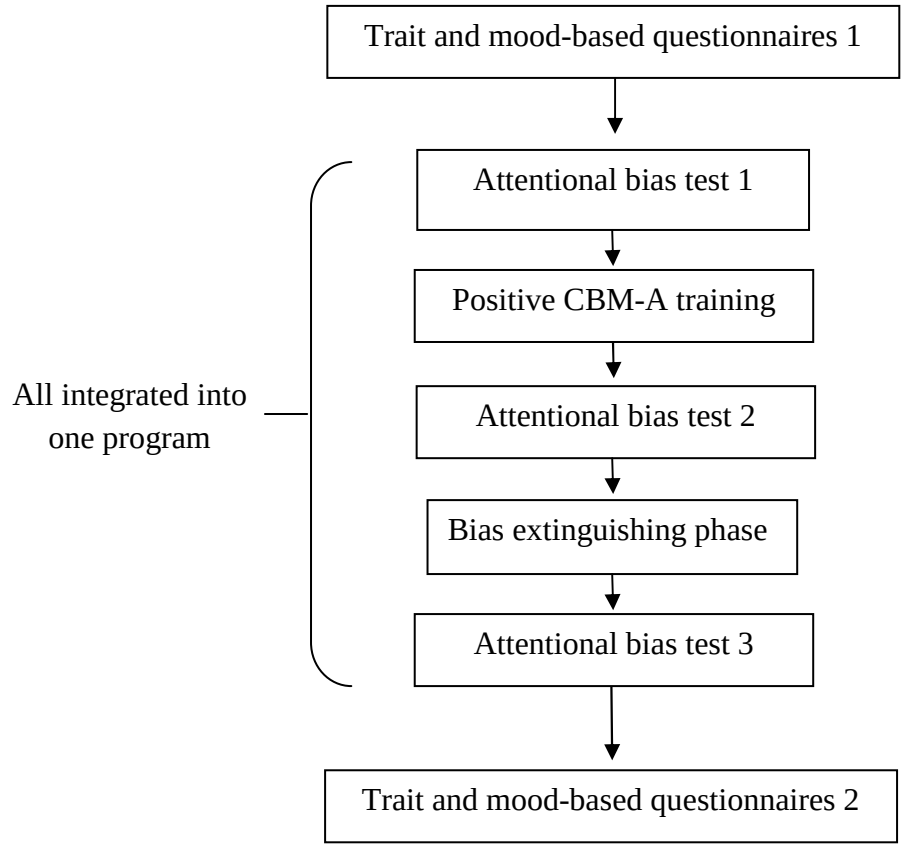

Figure 23. Overview of Study 6A's experimental design

8.1.1.3 Materials

CBM-A test/train program. The CBM-A test/train program was carried out on a Windows computer with the aid of E-Prime software (Schneider, Eschman, & Zuccolotto, 2002). The program consisted of (a) an initial attentional bias test (a visual probe task; Macleod, Mathews, & Tata, 1986), followed by (b) a positive attentional bias training phase (adapted from MacLeod et al., 2002), (c) a second bias test, (d) an 'untraining' phase, and (e) a final bias test. In its entirety, the program took approximately 30 minutes to complete. Each bias test consisted of 96 trials, whilst training and untraining phases consisted of 192 trials each. There were six scheduled breaks throughout the program, the length of which was determined by the participant.

For each trial participants had to respond to a target on a computer screen. Participants were initially presented with a fixation point in the centre of the screen, a "+" symbol, which then disappeared and was replaced by two words, one above and one below the location of the original fixation point. One of the words was always semantically neutral/positive whilst the other was always negative, although the positioning of the two words (either above or below the fixation point) was randomly selected by E-Prime. Both words disappeared after 500ms and either one or two dots (the target) appeared in the place of one of the words. Participants were required to identify whether there were one (".") or two ("..") dots present by pressing the *z* key or the *m* key on the keyboard, which were labelled as "1" and "2" respectively. For the bias tests and untraining trials the dots were positioned behind the positive and the negative words with equal frequency. However for the training trials the dots were always positioned behind the neutral/positive word.

Each CBM test/train program was counterbalanced using four word lists that were matched in terms of emotionality rating. Each list contained 12 words. Word lists were rotated so that every word list was used both to train and untrain a bias and test a bias for different participants. This counterbalancing technique completed a full rotation after every eighth participant. Participants were assigned numbers according to their entry to the study on a first come first served basis. This number determined which CBM test/train program the participant would be presented with according to the counterbalancing schedule described above. The computer program started with test one, for which participants were presented with 100% unseen word pairs. This was followed by the training phase, which was made up of words used in the first test (50%) and words from a new unseen list (50%). The second test then consisted of half of the word pairs from test 1 and half words from a so-far unseen list. The untraining phase used exactly the same words as in the training phase but in a different

order. Finally, test 3 was similar to test 2, with the same 50% of words sourced from test 1 and half unseen words.

Questionnaires. Both before and after completing the CBM test/train computer program, participants completed the State Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), and the Positive and Negative Affect Scale (PANAS; Watson et al., 1988). Both of these measures are described in Studies 1 and 2 respectively.

8.1.1.4 Procedure

Ethical approval was obtained from the School of Social Work and Psychology Ethics Committee at the University of East Anglia. Participants were paid £6 to recompense their time. Each session was run in groups of up to 15 participants in a computer laboratory on campus, where each participant could sit at an individual computer desk. Participants were welcomed into the study and issued with an information sheet which they were asked to read through. Once any questions were answered, and a consent form signed, the researcher read through an outline of the study in view of the fact that, once started, participants would most likely work through the session at different paces. Participants were asked to start by completing the first three questionnaires in their booklet, before completing the computer task. Participants were told that the computer task consisted of written instructions and a few practice trials before the main task, and were informed of the probable time taken to complete the task in total. After completing the computer task, participants were asked to complete the final three questionnaires and then alert the researcher that they had finished the study. The researcher then collected their paperwork and issued them with a debriefing sheet and £6 compensation for their time and effort. Participants were permitted to leave the room once they had finished, with overall session time ranging from between 35 and 45 minutes.

8.1.1.5 Data Analysis Plan

Data was explored to check it met the assumptions for parametric testing. Repeated measures ANOVAs were used to monitor changes in mood throughout the study. To test the study hypothesis, a series of repeated measures ANOVAs were conducted on bias test index data. Post-hoc testing was carried out using paired *t*-tests with Bonferroni correction where appropriate.

8.1.2 Results

8.1.2.1 Participant Characteristics

Neither trait, $F(1, 35) = 1.32$, $p = .26$, $\eta_p^2 = .04$, nor state anxiety, $F(1, 32) = .13$, $p = .73$, $\eta_p^2 < .01$, was found to change throughout the study. Negative affect was also found not to change significantly, $F(1, 38) = 1.08$, $p = .31$, $\eta_p^2 = .03$. However positive affect was found to significantly decrease over time, $F(1, 38) = 48.83$, $p < .001$, $\eta_p^2 = .56$ (see Table 20).

Table 20

Descriptive data for participants across the study

	Time 1		Time 2	
	Mean	SD	Mean	SD
Trait anxiety	44.22	10.26	43.69	11.33
State anxiety	37.15	10.49	37.67	8.29
Positive affect	30.18	6.31	25.49	8.59
Negative affect	14.49	5.51	13.85	3.54

8.1.2.2 Data Cleaning

Individual trials for which participants failed to correctly identify the probe were removed from analysis (3.77% total data: test 1 = 4.27%, test 2 = 3.22%, test 3 = 3.81%), as were trials with a reaction time of less than 200 milliseconds or greater than 2000 milliseconds (a further 0.20% total data: test 1 = 0.28%, test 2 = 0.55%, test 3 = 0.28%) in line with previous research (e.g. MacLeod et al., 2002; Koster, Crombez, Verschuere, & De Houwer, 2004). Overall, this meant 3.97% of the total data was removed from analysis (test 1: 4.54%, test 2: 3.28%, test 3: 4.08%).

8.1.2.3 Training Effects

A one-way repeated measures ANOVA was run with time (test 1: pre-training using ABI scores from all words; test 2: post-training using ABI scores from 'old' words[11] only; test 3: post-untraining using ABI scores from old words only) as a within subjects variable. There was no significant main effect of time, $F(2, 76) = .58$, $p = .57$, $\eta_p^2 = .02$, thus suggesting the training was ineffective in improving ABI scores (see Table 21). A repeated measures ANOVA was conducted with time (test 1: pre-training, all words; test 2: post-training, 'new' words; test 3: post-untraining, new words) as a within subjects factor to check whether training had been effective in improving ABI scores for previously unseen word pairs. Again, no significant main effect of time was found, $F(2, 76) = .53$, $p = .59$, $\eta_p^2 = .01$. This second finding is logical, given that training was found to be unsuccessful in making participants quicker to respond to probes that are placed behind positive words (which would be indicated by a higher positive ABI score) for 'old' words, that were used during test 1 and training. As this indicates that the training was ineffective, it would therefore be unlikely that effects of training would be seen to generalise to 'new' word pairs that had not previously been used in test or training trials.

[11]'Old' word pairs are words that have been presented to the participants before during training/untraining. 'New' words will forthwith refer to word pairs that have not previously appeared in the training/untraining and, as such, are novel to the participant. 'All' words will forthwith refer to a combination of 'new' and 'old' words within a word list.

Table 21

Mean (and SD) Attentional Bias Index scores for Tests 1, 2, and 3

		Test 1	Test 2	Test 3
All word pairs	Mean	-4.64	-2.19	.53
	SD	*17.73*	*23.86*	*19.15*
Old word pairs	Mean		-0.71	2.62
	SD		*32.20*	*33.65*
New word pairs	Mean		-9.71	-1.95
	SD		*51.26*	*21.91*

Note. Lower numbers indicate a more negative bias and higher numbers indicate a more positive bias.

In a recent study, Amir, Taylor, and Donohue (2011) found that baseline measures of attention bias were predictive of how receptive individuals were to an attention modification program. Participants who started with a more negative attention bias were found to be more responsive to training and were found to show better improvements in generalised social phobia symptomology. For this reason, it was decided to further look at the range of baseline (test 1: pre-training) bias scores before drawing any firm conclusions regarding the efficacy of CBM-A training.

8.1.2.4 Post-hoc Group Allocation

In the sample as a whole, bias scores ranged from -40.5 to 36.5 with a median score of -4.50 (M = -4.64, SD = 17.73). Due to the broad range of natural ABI scores, and in light of Amir et al.'s (2011) finding, it was decided to retrospectively divide participants into positive and negative bias conditions based on a median split. Following this division, 20 participants

were placed in the negative bias condition and 19 participants were placed in the positive bias condition[12].

There was no difference between the two groups in terms of trait anxiety, $F(1, 38) = .23$, $p = .64$, $\eta_p^2 = .01$, positive affect, $F(1, 39) = 1.30$, $p = .26$, $\eta_p^2 = .03$, or negative affect, $F(1, 39) = .59$, $p = .45$, $\eta_p^2 = .02$, on entry to the study. There was also found to be no influence of condition allocation on change in these variables throughout the study (all p values > .13). Participants with a negative bias were, however, found to have significantly higher levels of state anxiety on entrance to the study, $F(1, 35) = 5.85$, $p = .02$, $\eta_p^2 = .15$. This was considered not to warrant cause for concern in light of the fact (as mentioned previously) that there was no change in state anxiety throughout the study, $F(1, 31) = .10$, $p = .75$, $\eta_p^2 = .003$, and no significant interaction between the two conditions and state anxiety throughout the study, $F(1, 31) = .10$, $p = .75$, $\eta_p^2 = .003$.

Effect and robustness of training. A 2 (group: positive, negative starting bias) x 3 (test 1: pre-training using all words; test 2: post-training using old words [previously used in test/training/untraining] only; test 3: post-untraining using old words) repeated measures ANOVA was conducted to determine whether post-hoc group allocation influenced training success. Whilst no main effect was identified in overall bias change over time, $F(2, 74) = .56$, $p = .57$, $\eta_p^2 = .02$, a significant interaction between group (whether participants started with a more positive or a negative ABI score) and time was found, $F(2, 74) = 5.24$, $p = .007$, $\eta_p^2 = .12$.

For participants starting with a positive bias, post-hoc testing in the form of paired *t*-tests was conducted between tests 1-2, 2-3, and 1-3. Comparison of tests 1-2 examined whether training had been effective, while comparison of tests 2-3 and 1-3 investigated

[12] It is acknowledged that this 'positive' and 'negative' starting bias is specifically relative to the overall range of the group, rather than a generic classification.

whether any effects of training endured the period of untraining. No significant change in ABI scores was found between tests 1-2, 2-3, or 1-3 for old (previously exposed), new (not previously used) words, or all (old and new) words (all *p* values > .10). However, a trend level of significance was observed between tests 1-2 when looking at all words, $t(18) = 2.36$, $p = .03$, $d = .79$ (Bonferroni corrected $\alpha = .017$). As illustrated in Figure 24, and in line with Amir et al.'s (2011) findings, this trend appears to show a less positive and more negative bias following the positive CBM-A training.

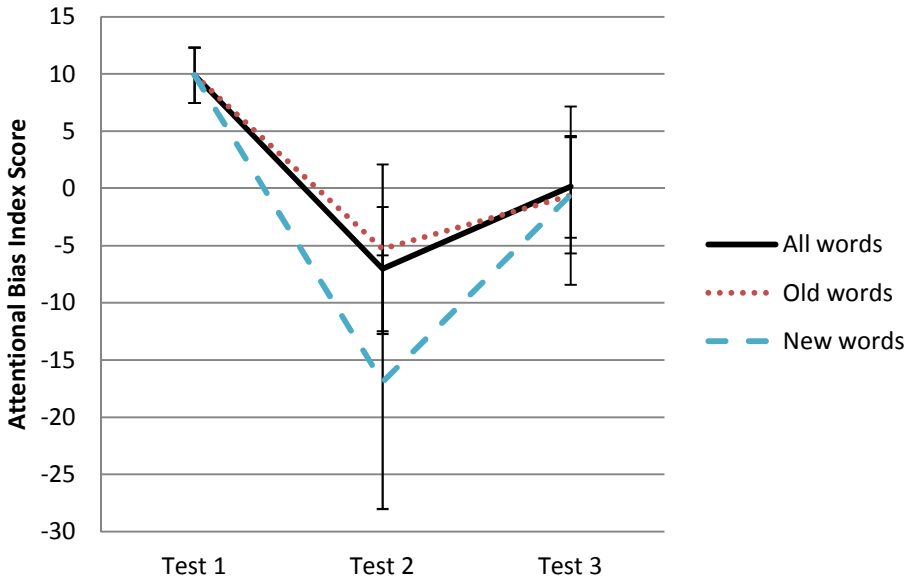

Figure 24. Mean change (and SE) in ABI score in participants starting with a positive bias

The same series of paired *t*-tests was conducted on ABI score data from participants who entered the study with a negative bias. In this sample, training was found to be successful in inducing a more positive bias when comparing all words at test 1 with old words at test 2, $t(19) = -3.44$, $p = .003$, $d = 1.19$ (Bonferroni corrected $\alpha = .0167$), improving mean ABI scores from -18.45 (*SD* = 11.06) to 3.65 (*SD* = 23.78). The same effect was also evident when looking at all words at test 1 versus all words at test 2 $t(19) = -4.10$, $p = .001$, $d = 1.40$, improving mean ABI scores from -18.45 (*SD* = 11.06) to 2.40 (*SD* = 17.92). This finding suggests that, considered together, training appears to successfully generalise onto

both old and new word pairs. The difference between all and new words at tests 1-2 was only found to approach significance in consideration of the Bonferroni correction, $t(19) = -2.23$, $p = .038$, $d = .71$, which implies that the training effects were stronger for word pairs that participants had been previously exposed to relative to novel word pairs. However, when comparing ABI scores using all-old, all-new, and all-all word pairs at tests 1-3, a significant difference was found between all comparisons (all p values < .016). Furthermore, the lack of any significant change between any of the three word type combinations from tests 2-3 (all p values > .26) suggests that such improvements in ABI scores are further maintained at test 3 and so appear to survive untraining (see Figure 25).

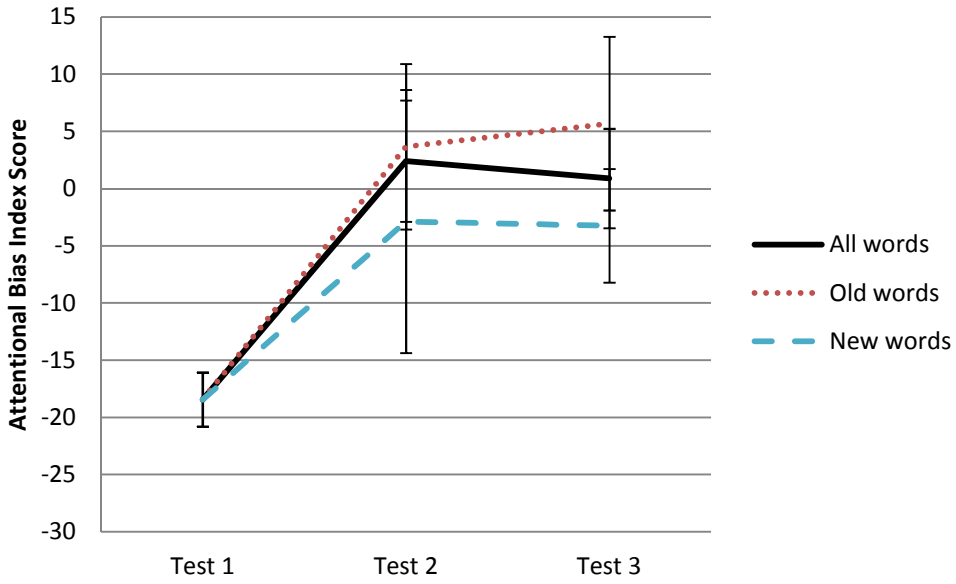

Figure 25. Mean change (and SE) in ABI score in participants starting with a negative bias

Summary

When considered as one group, there was no effect of CBM-A training. This was counter to what was hypothesised. However, once retrospectively divided according to a median split of entering ABI scores, CBM-A training had the hypothesised significantly positive effect on participants who started with a more negative bias, which was maintained

after untraining. Alternatively, and in contrast to the hypothesis, participants starting with a more positive bias showed no CBM-A training effect.

8.2 STUDY 6B

8.2.1 Method

8.2.1.1 Design

This study employed a repeated measures design as all participants completed the same test/train procedure (see Figure 26). Participants completed three CBM-I tests throughout the session, pre-training, post-training, and post-untraining (independent variable). The dependent variable was the reaction time taken to solve negatively valenced or neutral associate word fragments, which was condensed into a single interpretive bias index (IBI) score. This was calculated in the same manner as for attentional bias index in Study 6a, by subtracting reaction time taken by participants to indicate they could solve the positive word fragment from that taken to respond to negative word fragments in the same respect. For the resulting IBI score, a larger positive number represents a stronger positive bias and a lower negative number represents a stronger negative bias.

8.2.1.2 Participants

Forty participants, composed of staff and students at the University of East Anglia, were recruited through the same techniques as used in Study 6a. All participants (27 females) were aged between 18 and 60. Participants entered the study with levels of trait anxiety averaging 33.03 (*SD* = 9.25).

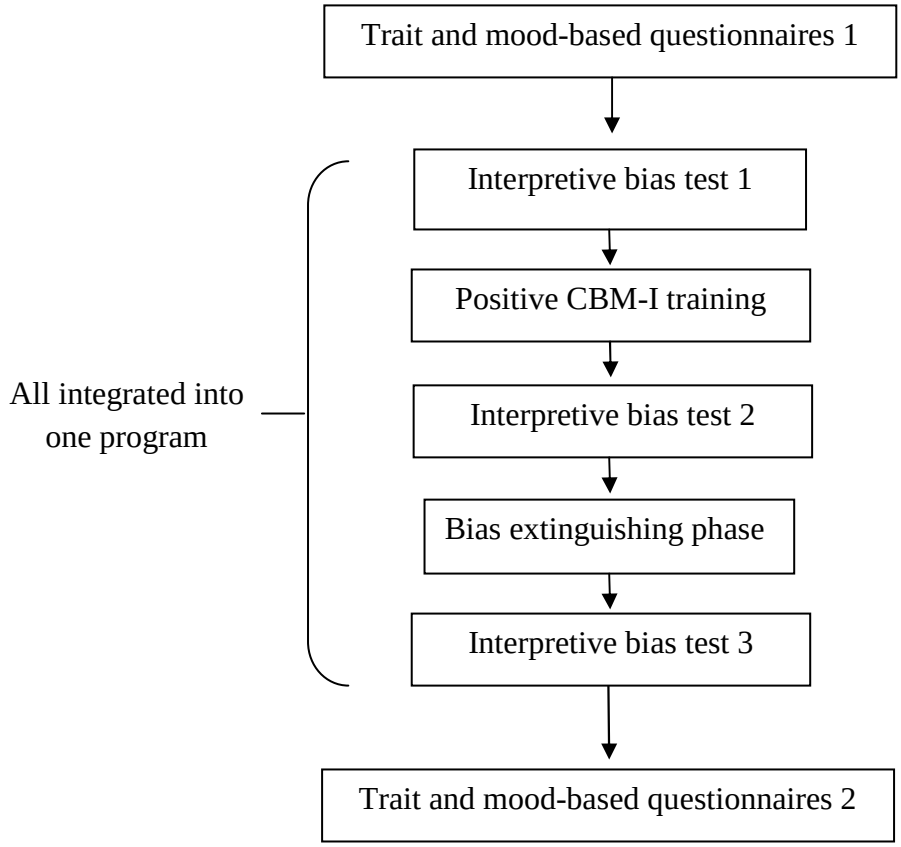

Figure 26. Overview of Study 6B's experimental design

8.2.1.3 Materials

CBM-I test/train program. As in Study 6a, the program was delivered with the aid of E-Prime software (Schneider, Eschman, & Zuccolotto, 2002) and consisted of (a) a baseline interpretive bias test (adapted from Grey & Mathews, 2000, using words from French & Richards, 1992) followed by (b) a positive interpretive bias training phase, (c) a second test, (d) untraining phase, and (e) final test. In total, participants took approximately 30 minutes to complete the program. Each bias test consisted of 32 trials, while training and untraining consisted of 64 trials, and the program consisted of several scheduled breaks.

For each trial, participants had to solve a word fragment that appeared after a clue word on the computer screen. Participants were instructed to use the clue word to help them

solve the word fragment. Each clue word was an emotional homograph; a word that has multiple meanings depending on the context within which it is used. All homographs were selected for having both strong neutral and an alternative strong negative interpretation. For example, the word *"arms"* might refer to the upper body limb (neutral) or to the process of equipping a person with weapons (negative). The clue homograph remained on screen whilst participants tried to solve the word fragment. Participants were told the clue word was designed to help them resolve the word fragment, though were not explicitly informed that all clue words were homographs. Participants were required to press the spacebar on the keyboard once they had resolved the word fragment, and were then instructed to locate and press the letter key that represented the first missing letter of the word fragment. For bias tests and untraining, positive and negative interpretations of homograph clue words were drawn on an equal amount of times. However, for positive bias training, the word fragment was consistently resolved into the positive associated meaning.

Each test/train program was composed of six word lists, each with 16 words that additionally had four possible positive and four possible negative associated word fragments, so that all word lists were used both as training and testing material. Each word list was matched in terms of emotionality ratings and word lists were counterbalanced across participants. As with Study 6a, participants were assigned numbers (which determined their counterbalanced rotation) on a first come first served basis.

For each participant, the first test was composed using 100% unseen clue words drawn from two of the six word lists. The positive training phase used 50% old (previously seen) clue words and 50% new words from two more word lists. The second bias test used 50% clue words from test 1 and 50% previously unseen words from the fifth list. Untraining used the same clue words that were used in positive training, though this time drawing on both positive and negative associations of the homograph. Finally, the third test used the

same 50% clue words that were used in the first two tests, and 50% new clue words from a final word list. Where a clue word was used more than once in the different phases of the program (for example, in tests 1, 2, and 3) different associate word fragments were used each time that were not necessarily of the same valence as on the previous occasion.

Questionnaires. As in Study 6a, participants completed the STAI and the PANAS both before and after completing the CBM test/train program. Further details on these scales can be found in Studies 1 and 2 respectively.

8.2.1.4 Procedure

Ethical approval was obtained from the School of Social Work and Psychology Ethics Committee at the University of East Anglia. Participants were recompensed with £6 for their time and effort. The procedure was primarily the same as in Study 6a, with the exception that the interpretive CBM test/train program being used in place of the program aimed at testing and training an attentional cognitive bias. As with Study 6a, participants were able to leave once they had finished the set procedure, which was typically after 35 – 45 minutes.

8.2.1.5 Data Analysis Plan

Data was explored and analysed in the same manner as in Study 6a.

8.2.2 Results

8.2.2.1 Participant Characteristics

As a group, state anxiety increased significantly throughout the study, $F(1, 38) = 5.10$, $p = .03$, $\eta_p^2 = .12$ (see Table 22). Positive affect significantly decreased over time, $F(1, 39) = 18.12$, $p < .001$, $\eta_p^2 = .32$. Neither trait anxiety nor negative affect changed significantly over the duration of the study (both p values >.20).

Table 22

Descriptive data for participants across the study

	Measure 1		**Measure 2**	
	Mean	SD	Mean	SD
Trait anxiety	40.89	9.33	40.21	9.73
State anxiety	33.03	9.25	35.80	9.03
Positive affect	32.50	7.40	29.25	8.60
Negative affect	13.45	3.35	13.08	3.06

8.2.2.2 Data Cleaning

Prior to the calculation of IBI scores, incorrect trials were removed from the analysis (comprising 15.5% data: 17.8% from test 1, 17.5% from test 2, and 11.2% from test 3). Filters were set on the remaining data to remove extraneous data, which consisted of trials taking less than 200 milliseconds (0.5% data: 1.0% from test 1, 0.4% from test 2, and 0.3% from test 3) or more than 6000 milliseconds (a further 1.9% data: 2.8% from test 1, 1.8% from test 2, and 1.1% from test 3) in accordance with similar action taken by Grey and

Mathews (2000). Overall, 17.5% data was removed due to the aforementioned reasons, comprising 20.9% from test 1, 19.3% from test 2, and 12.3% from test 3.

8.2.2.3 Training Effects

To test the efficacy of training, a repeated measures ANOVA was run using time of interpretive bias test (test 1: pre-training using all homographs; test 2: post-training using previously seen 'old' homographs[13]; test 3: post-untraining using old homographs) as a within subjects factor. A significant main effect of time was found, $F(2, 78) = 16.03$, $p < .001$, $\eta_p^2 = .29$, which indicated that training might have been effective in training a more positive bias.

Paired *t*-tests were carried out to further investigate the main effect. As can be seen from Table 23, looking at comparisons between tests 1 and 2, training appeared effective when comparing either all homographs or old homographs at test 2 but not when using new homographs at test 2. This suggests that, while training is effective, the effects have not completely generalised to new stimuli. Comparing tests 2-3, when looking at new homographs for both tests there is no significant change, suggesting that training effects remain absent in these homographs. Significant decreases in IBI scores are evident when looking at either all-all or old-old homographs for tests 2-3, which implies that the effects of training did not endure untraining. This is supported by the fact that comparisons between IBI scores at test 1 (all homographs) and tests 3 (all, old, or new homographs) show no significant difference (see Figure 27).

[13] As with Study 6a, 'old' homographs refers to those that have previously been used in tests or training/untraining, 'new' homographs refers to those that have not been used in previous tests or training/untraining, and 'all' refers to both 'old' and 'new' combined.

Table 23

The difference between IBI scores over time according to homograph familiarity

		Test 2 (post-training)						Test 3 (post-untraining)					
		All		**Old**		**New**		**All**		**Old**		**New**	
		t	***p***	***t***	***p***	***t***	***p***	***t***	***p***	***t***	***p***	***t***	***P***
Test 1	**All**	-3.41	.002	-5.16	<.001	-.60	.55	.11	.91	-1.80	.08	1.61	.12
Test 3	**All**	3.82	<.001										
	Old			3.78	.001								
	New					2.26	.03						

Note. All, Old, and New refers to the word lists that the homographs originated from. Bonferroni corrected α = .017.

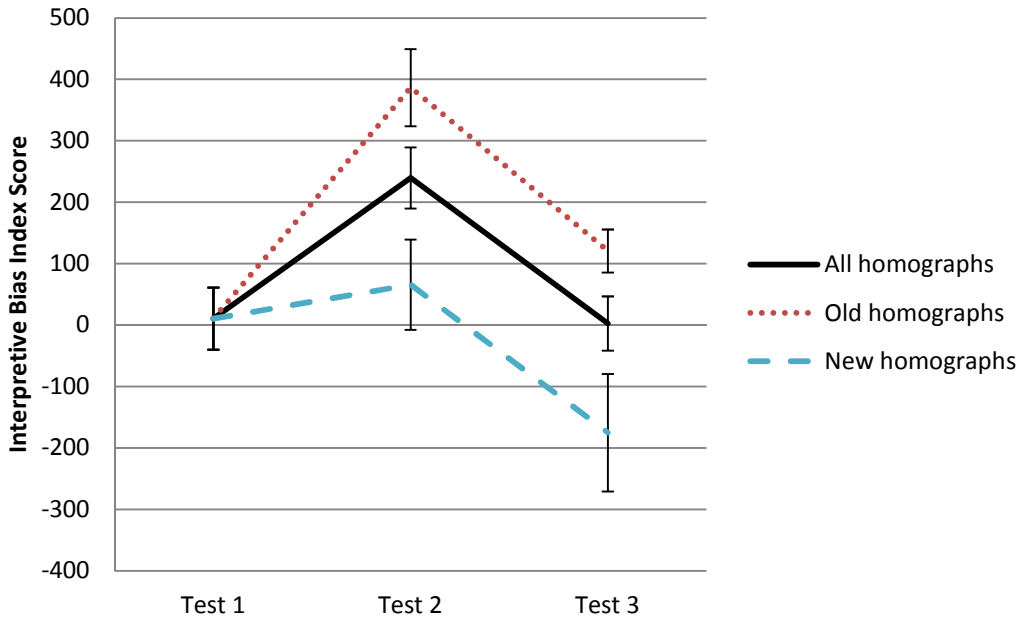

Figure 27. Mean change (and SE) in IBI scores in all participants

8.2.2.4 Post-hoc Group Allocation

In an attempt to replicate the results from Study 6a using attentional bias training, a decision was made to split participants retrospectively into conditions based on their starting bias; those starting with a more positive bias (any score above the median of -14.56) and those starting with a more negative bias (any score under -14.56). There was no difference between the two conditions in terms of entry state anxiety, $F(1, 37) = .18$, $p = .67$, $\eta_p^2 = .01$, trait anxiety, $F(1, 37) = .07$, $p = .80$, $\eta_p^2 < .01$, positive affect, $F(1, 38) = 1.05$, $p = .31$, $\eta_p^2 = .03$, or negative affect, $F(1, 38) = 1.29$, $p = .26$, $\eta_p^2 = .03$. There was no significant interaction between condition allocation and change in state anxiety ($p = .23$) or negative affect ($p = .94$). However the interactions were approaching significance for change in trait anxiety ($p = .08$) and positive affect ($p = .06$). Further analysis revealed no significant change in trait anxiety for participants starting with a negative or positive IBI score (both p values > .12). For participants starting with a positive bias, positive affect significantly decreased from a mean of 33.70 ($SD = 7.50$) to 29.00 ($SD = 8.98$), $F(1, 19) = 17.70$, $p < .001$, $\eta_p^2 = .48$. There was no change in positive affect for participants starting with a negative bias, $F(1, 19) = 3.51$, $p = .08$, $\eta_p^2 = .16$.

Training effects following group allocation. A 2 (between-subjects factor; group: more positive starting bias or more negative starting bias) x 3 (within-subjects factor; time of CBM-I test: pre-training using all homographs, post-training using old homographs, and post-untraining using old homographs) mixed model ANOVA was conducted. As before, there was a significant main effect of time on change in IBI score[14], $F(2, 76) = 10.68$, $p < .001$, $\eta_p^2 = .23$. A significant time x group interaction was also identified, $F(2, 76) = 13.27$, $p < .001$,

[14] Main effects are not further discussed here as they remain the same as before, see 'Training effects' subsection of this results section.

η_p^2 = .26. The same statistical tests were run using new homographs at tests 2 and 3, and all homographs at tests 2 and 3, with the same results (all *p* values <.01).

For participants starting with a positive bias, further investigation in the form of paired *t*-tests revealed no significant change in IBI scores between test 1 and 2, when focusing on all, new, or old homographs at test 2 (all *p* values > .14). A significant decrease in IBI score was identified from tests 2 – 3 when focused on 'all' homographs, $t(19) = 3.33$, $p = .004$, $d = .88$ (Bonferroni corrected $\alpha = .017$), and old homographs, $t(19) = 3.35$, $p = .003$, $d = .94$. This suggests that the *positive* CBM-I training led to participants showing a *reduction* in IBI scores, indicating there were adverse effects of training. When focusing on the difference in IBI scores on new homographs at test 2-3, the corrected significance level was not reached, $t(19) = 2.32$, $p = .03$, $d = .54$. A significant decrease was also identified when comparing IBI scores obtained at test 1 with those obtained at test 3 for every word list (all *p* values < .017; see Figure 28).

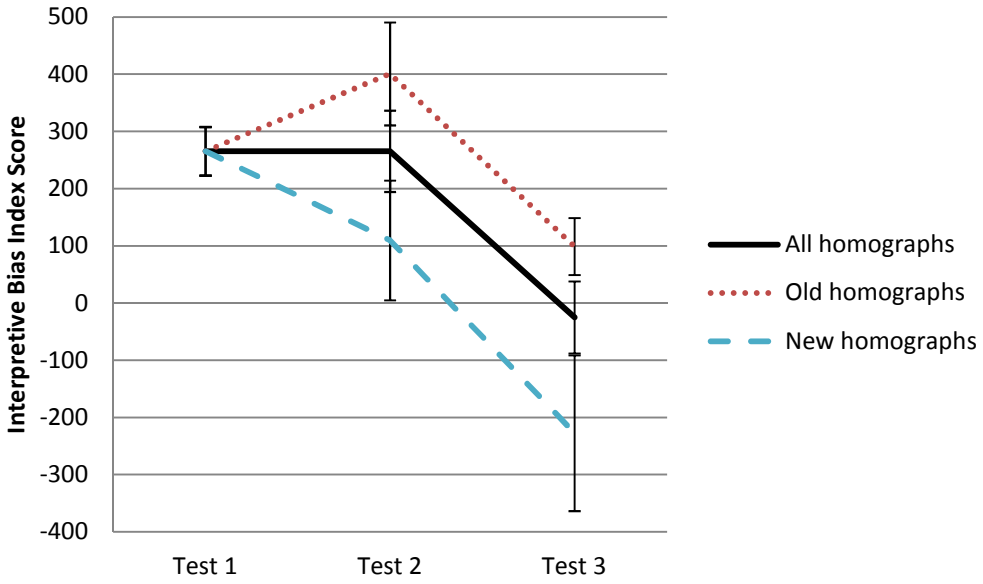

Figure 28. Mean IBI score change (and SE) in participants starting with a positive bias

Alternatively, for participants starting with a negative bias, further analysis revealed a significant increase in IBI score from tests 1-2 when looking at old, $t(19) = -6.89$, $p < .001$, $d = 1.94$, new, $t(19) = -2.66$, $p = .016$, $d = .96$, or all, $t(19) = -6.99$, $p < .001$, $d = 2.17$, homographs at test 2. This indicated that training had the expected effect of improving IBI scores. There was no significant change between any of the word lists from test 2 – 3 (all p values > .05). A significant improvement in IBI scores at test 3 compared with test 1 for old homographs, $t(19) = -6.12$, $p < .001$, $d = 2.16$, and 'all' homographs, $t(19) = -3.31$, $p = .004$, $d = 1.20$ was found. However no change was observed between the two tests for new homographs, $t(19) = -.90$, $p = .38$, $d = .31$ (see Figure 29).

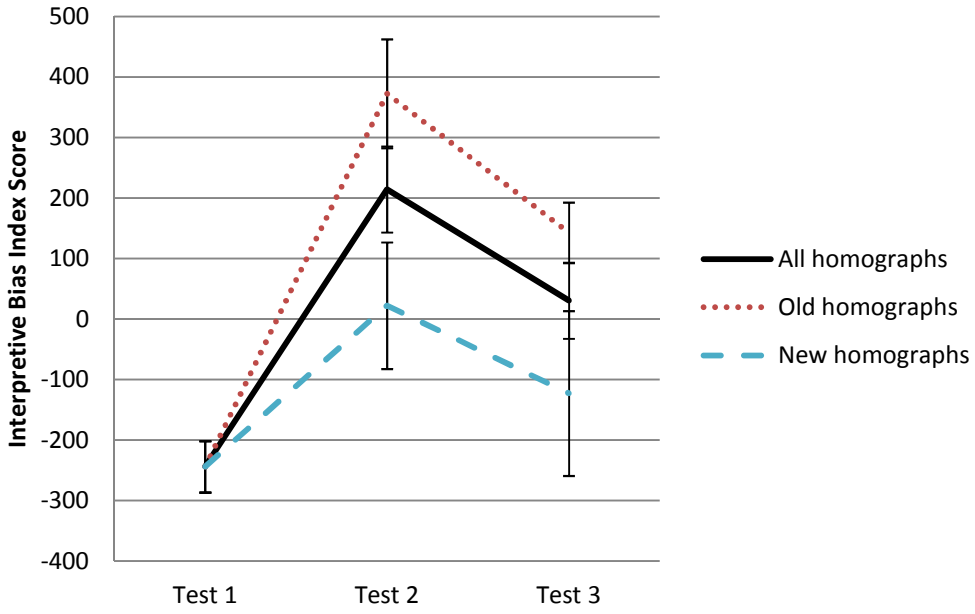

Figure 29. Mean IBI score change (and SE) in participants starting with a negative bias

Summary

When considered as one group, training appeared to be effective when looking at all or old homographs at test 2. However there were no training effects for new homographs at test 2, suggesting that the training had not generalised fully. Further, the training effects for all and old homographs appeared to extinguish following untraining. A post-hoc split according to starting interpretive bias (more positive or more negative) revealed some

potential negative effects of training when participants started with a positive bias. Participants starting with a negative bias, alternatively, appeared to benefit from training with the effects enduring untraining.

8.3 Discussion

This study sought to identify whether an acute period of 'untraining', that is, a series of trials that mimicked training in structure and length but had no contingency between the positive emotive word (CBM-A) or homograph interpretation (CBM-I) and the target (CBM-A: dot probe; CBM-I: associated word fragment), had any effect on a freshly positive-trained bias. For attentional bias, initial analysis suggested that the hypothesis was not supported. Results indicated that training had been ineffective, as there was no increase in ABI scores either on trials that included word pairs that had been previously seen (old) or those that were new (new). In consideration of findings from a recent study suggesting that an individual's initial bias was able to moderate their receptiveness to training (Amir, Taylor, & Donohue, 2011), the data was subjected to a post-hoc median split.

In line with Amir et al. (2011), CBM-A training was found to be ineffective in participants who had started with a stronger positive bias relative to the group. Further, indications from the mean and a trend effect suggested that training was starting to have adverse effects, as ABI scores were lower at the second (post-training) attentional bias test in this sub-group. Alternatively, participants who had a relative negative starting bias showed significant increases in ABI scores between tests 1 and 2, and no change between tests 2 and 3, which is taken to signify that training was effective and that the effects endured untraining.

When considered as one group, participants who underwent CBM-I training did partially appear to show predicted effects of training, as interpretive bias index scores significantly increased from tests 1 – 2 when looking at homographs that participants had been previously exposed to (old) or all homographs (all) at test 2. There were no significant effects of training when looking solely at homographs that were new to the participant at test 2 (new), suggesting that the effects were not strong enough to generalise to new material.

Further, the training effects found in old and all homographs at test 2 appeared to be extinguished by untraining by test 3.

Again exploring notions stemming from Amir et al.'s (2011) findings, the sample from experiment 6b was also subjected to a median split based on starting bias. Results supported findings from Study 6a, as participants starting with a (relative) negative bias showed positive effects of training that were maintained through the period of untraining while participants starting with a (relative) positive bias showed no effects of training. Further, this subgroup showed significant decreases in IBI scores between tests 2-3 and 1-3, suggesting that the procedure may adversely affect interpretive bias.

The post-hoc median split findings from both experiments support Amir et al.'s (2011) study and posit that a participant's natural bias should be considered before rendering them suitable for CBM training. Evidence of these patterns of response are of critical importance, as it has previously been unprecedented to conceive of a notion that there would be situations for which CBM might not be suitable or individuals for whom CBM training might have an adverse affect. The results from the two experiments presented here, in addition to those from Amir et al., suggest that CBM might be less a case of generic help and more a directed cause for repair where damage exists.

It is acknowledged that participants in the current studies were categorised as having a more positive or a more negative starting bias according to a median split of bias index scores in each of the study samples. It remains possible that there might be some common cut-off according to bias index scores, above which training would always be ineffective or negatively effective. It seems plausible, at least, that there exists some looser form of class determining suitability according to bias index score, which might better define who would be best suited to CBM. It is further likely that the absence of these patterns of response in

previous work demonstrating the potential of CBM in a clinical setting (e.g. Amir et al., 2009) can be attributed to the participant sample that was recruited. For example, Amir et al. focused on participants who had been diagnosed with generalised social phobia symptomology. In consideration of the strong inverse link between anxiety and cognitive bias (e.g. Eysenck, MacLeod, & Mathews, 1987), it is likely that such a sample would have naturally all had strong negative biases. For this reason all participants might arguably have inadvertently fallen below this theoretical threshold of suitability and so would all have been receptive to the positive effects of CBM training.

It is possible to cautiously apply some of the logic assembled from the present findings to the results of Study 5. In Study 5, which was conducted before the present two experiments and before the publication of Amir et al.'s (2011) study, there was no consideration made to natural bias and individual suitability to CBM-I training; all participants received either sham or positive CBM-I training. Following training, participants then completed an interpretive bias test to check whether training had been successful. Statistical testing did confirm this, with participants in the positive CBM-I training group having a significantly more positive bias than participants in the sham CBM-I training group. However, though statistically significant, the effect size was small (.07). This could arguably be due to the finding from the present experiments that participants do not all respond to training in a uniform manner. For example, by training all participants regardless of their starting bias, some participants (who had a starting negative bias) might have been more receptive to positive CBM-I training while others (who had a starting positive bias) might have been less receptive to positive CBM-I training.

Results from Study 5 suggested that participants who reported high levels of test anxiety showed an improved cortisol recovery to the test stressor following positive CBM-I training relative to sham CBM-I training. Alternatively, participants who reported low test

anxiety who received positive CBM-I training showed poorer sAA recovery to a test stressor relative to low test anxiety individuals who received sham CBM-I training. In hindsight, it is possible to cautiously assume that those individuals who had high test anxiety might plausibly also have had a naturally occurring stronger negative bias relative to those who had low test anxiety. In line with the current experimental findings, it could therefore be proposed that those participants who had a stronger negative bias (inferred from having higher test anxiety) were more suited to the positive CBM-I training group than those who had a stronger positive bias (inferred from having lower test anxiety). Further, those with a stronger positive bias (low test anxiety) appear more suited to the sham CBM-I training group than those with a stronger negative bias (high test anxiety).

The present study adopted a fairly rudimentary methodology with regards to the positioning of the untraining period immediately after the training period. Future research might seek to investigate a larger timeline of the enduring effects of training. For example, participants might undergo a more intense schedule of CBM training on one day, week, or month followed by a similarly intense session of untraining the next. Alternatively, studies might look to interchange daily episodes of training/untraining a bias to determine whether any accumulating effects of training are able to develop when training sessions are interrupted. This might help researchers to better understand the individual features that make training successful, which could serve to strengthen the impact of training in terms of its clinical potential by improving guidelines relating to CBM training. Further, it would be worthwhile to include measures of stress physiology in future more long-term research, to investigate the influences on psychophysiology.

In conclusion, the present study has found evidence to suggest that CBM training might not be generically suited to all but more specifically suited to those who need it. For individuals to whom training is suitable, one session of either attentional or interpretive

training was found to be successful in improving bias with the effects generalising to new material. These effects were found to be robust enough to endure a session of untraining that was presented immediately afterwards. For individuals to whom training appears unsuitable, evidence emerged to suggest that positive training might adversely affect bias. These results seem promising in terms of the methods' potential for the use in a clinical setting, although caution should be issued to future research studies that use a control group who are considered to have 'normal' levels of anxiety. Further research is needed to understand the nature both of who might be suited to CBM and also of the conditions under which CBM might be most effective. As a clinical tool, the current study does support the broad literature suggesting its potential, though much work is needed prior to its release as an alternative to more conventional therapies.

9.0 CHAPTER NINE

GENERAL DISCUSSION

Previous research had found evidence for a relationship between cognitive bias and the perception of stress. However, there has been little systematic attempt made to understand whether cognitive biases also relate to the biological response to stress. If, as hypothesised, the biological response to stress is linked to cognitive bias then biases hold the potential to predict both an individual's feelings about stress and also the manners in which the brain communicates stress to the body. This has potentially profound implications for the long term health of individuals with negative biases.

In view of the critical importance of reliably measuring the biological response, two studies (Studies 2 and 3) researched the optimal methods and practices of collecting saliva samples. The results of these two studies highlighted gender differences and fed into the studies that examined the relationship between bias and the physiological stress response.

Studies investigating the bias/stress response relationship (Studies 1, 4, and 5) encountered unexpected difficulties in eliciting psychological and biological stress responses. Where a stress response was induced, there was limited evidence to support previous research demonstrating a robust link between attentional bias and emotional vulnerability to stress (Study 4), and no suggestion of influences on a biological scale. Similarly, interpretive biases were not shown to strongly moderate psychological responses to acute challenges (Study 4), and positive CBM-I training did not serve to buffer subsequent exposure to stressors (Study 5). However, interpretive biases did appear to moderate the biological recovery process, and positive CBM-I training was found to lead to a more efficient biological recovery following acute stress relative to sham training in high test-anxious individuals. Importantly, for both CBM-I and CBM-A, evidence emerged to suggest that training techniques might in some

instances lead to negative effects. For participants who had low levels of trait anxiety (Study 5) or a natural positive bias (Study 6), positive training appeared to lead to a slower biological recovery to stress and either no change or a significant decrease in bias index scores.

These findings will be discussed in terms of their original contribution to the field, the implications of such findings regarding the clinical potential of CBM techniques, limitations of the research, and directions for future research. Prior to this, studies will be briefly summarised to remind the reader of their individual aims and outcomes.

9.1 Summary of Studies

Study one. With the aim of establishing a reliable social rejection stressor paradigm, this study adopted a protocol that was adapted from Blackhart, Eckel and Tice (2007), who reported a significant cortisol response. Social rejection was induced in female participants by making them believe that no person in a group (up to 4 individuals) wanted to partner them for a group exercise. The study did not show evidence of an ANS physiological stress response, with no change being found in the rate at which sAA is secreted. Further, social rejection appeared to lead to a significant decrease in cortisol concentration relative to the comparison (social inclusion) condition. Psychological variables also largely showed no significant change in response to the intended stressor. Participants in the social rejection group reported no change in their perceived stress (as indexed by the SACL). Measured through a visual analogue scale, reported optimism and happiness were found to decrease following social rejection, though reported levels of tension and distress remained unchanged. Overall, the study was unsuccessful in its aims to replicate a biological and psychological stress response using Blackhart et al.'s social rejection paradigm.

Study two. This study was developed in response to observing some odd physiological patterns in Study 1, specifically finding consistent and unexpected changes between the salivary flow rate and analyte levels in the first two saliva samples. Study 2 examined whether a practice saliva sample was necessary to increase the validity and reliability of the first 'real' sample given, which often forms all or part of the crucial baseline analyte data. Two groups of participants practiced saliva donation using the passive drool technique (once or three times), and one group were afforded no practice sample. Participants then all gave four saliva samples, from which flow rate was calculated and assays conducted to determine levels of cortisol and sAA. As predicted, cortisol was unaffected by whether or not participants had practiced the technique. A significant main effect of time was identified for variation in flow rate, with an increase in sample volume being found between samples one and two. This main effect was further qualified by a significant three way interaction between flow rate, group allocation, and gender. Exploratory investigations revealed the hypothesised "practice effect" in female participants who had not practiced the technique, evidenced by a significant increase in flow rate between the first two samples. This effect remained absent in samples from female participants who had practiced the collection method either once or three times, and in males entirely. There was no evidence of any practice effects in sAA activity, which was unexpected given the observed findings in flow rate, and, as expected, no change in sAA output. Overall, Study 2 found evidence to suggest that, to err on the side of caution, research protocols that recruit female participants and collect saliva would benefit from implementing practice samples.

Study three. In a bid to establish an optimal procedure for saliva collection, Study 3 sought to compare two common methods used in biobehavioural research to collect saliva; passive drool into a cryovial and collection using a Salimetrics Oral Swab (SOS). Participants gave a sample using both methods (counterbalanced). No significant difference was found in

flow rate or sAA activity or secretion between the two methods. Cortisol was found in significantly increased amounts when samples were collected using the SOS relative to the passive drool method. Even so, significant correlations were found between the two methods for flow rate, cortisol, and sAA activity and secretion. Further, drawing on the practical concerns associated with working with saliva, the absorbent swab from the SOS acted as a filter for sample debris, resulting in a cleaner sample. This significantly enhanced the utility of low volume samples, which can otherwise be deemed unusable. Taken together, these findings led to a decision to favour saliva collection through SOS absorption relative to passive drool.

Study four. Adopting the methods and procedures developed in Studies 2 and 3, Study 4 examined the predictive capacities of natural interpretive and attentional biases on psychophysiological responses to an acute stressor (social ostracism) task. Implementing some recommendations arising from Study 1, Study 4 adopted an alternate stressor design in which participants were unexpectedly ignored during a 2 minute presentation of a neutral/positive topic to two (confederate) participants via a video conference link. Conferences were in fact artificial, with pre-recorded videos replacing real-time interactions. Participants took part in two of these staged interactions. During the first one, confederates assumed a neutral role, whilst in the second they acted in a way to induce positive or negative reactions. Positive reactions were induced through smiling, leaning in to the camera, and nodding. Negative reactions were induced by disengaging from the participant's presentation and whispering between themselves. Interpretive and attentional bias was measured prior to the stressor, and was used to try and predict variation in psychological and physiological measures.

Indices of psychological well-being indicated the task was stressful, with an increase in feelings of social rejection (from specific items embedded in the PANAS) and state anxiety

(STAI-s), as well as a reduced fulfilment of primary needs. Condition allocation (social ostracism, social inclusion) was found to significantly predict variation in psychological measure; stress (SACL), and positive and negative affect (PANAS). However, condition allocation did not predict changes in cortisol or sAA secretion. Attentional bias was identified as a trend predictor for changes in negative affect (PANAS) in response to the process of self-presentation (OCam 1 - neutral), though was not predictive of other psychological or physiological responses to the task, and held no predictive power for responses to social ostracism or recovery. Interpretive bias emerged as a near significant predictor for variation in reported stress (SACL) in response to the task (OCam 1 - neutral), and was also found to be a trend predictor for variation in negative affect in socially included participants only. While interpretive bias was not found to predict psychological or physiological responses to social rejection, it did appear to be a trend predictor of sAA recovery to the process of self-presentation.

Overall, Study 4 found little evidence to support existing literature that suggests a robust link between attentional and interpretive biases and emotional vulnerability, and did not replicate recent investigations on the physiological stress system and bias that have documented responses to the same effect. Of interest, however, interpretive bias was here found to influence recovery from acute stress.

Study five. Study 5 explored the effects of CBM-I on the psychophysiological stress response. A decision was made to use a stressor task that had been developed in parallel to the research presented in this thesis that focused on performance stress. Justification for this change in direction arose from the fact that the new paradigm had successfully been shown to act as an acute stressor, had been used to demonstrate clear links between bias and the stress response, and was sensitive to CBM-I techniques. Participants completed a session of CBM-I training using ambiguous vignettes (70 scenarios). Participants then completed a recognition

test before taking part in the stressor, which incorporated academic and social themes. In groups of up to 12, participants were instructed to complete three short computer tests, and received deceptive information specifying the nature of the tests (that they measured intelligence) and difficulty (that participants should not encounter problems in completing the tasks). The three programs were designed to be extremely challenging. Participants were also informed that their performance would be displayed publicly at the front of the room and that they might have to comment on their score should they perform particularly well or poorly.

Questionnaire measures (SACL, PANAS, and STAI-s) appeared to confirm that the task was acutely stressful, though no significant interaction was found between changes in these psychological measures over time and CBM-I condition (sham or positive training). As in Study 4, this finding appeared to contradict previous studies that have documented a reduced psychological vulnerability to stress following CBM-I training. Test anxiety appeared to influence psychological responses to the stressor, with higher test anxiety being associated with a larger psychological response relative to lower test anxiety. Cortisol appeared unaffected by the stressor, though again test anxiety was found to significantly interact with reactivity. A significant decrease in cortisol was identified following CBM-I training, which appeared steeper for low anxious individuals when they were in the sham training group and for high anxious individuals when they were in the positive training group. Further, high anxious individuals showed a faster cortisol recovery from the stressor only when they had received positive CBM training as opposed to sham training. Alternatively, sAA was found to significantly increase following CBM training, remain unchanged following the stressor (though changes were in the predicted direction), and then recover following the stressor. As with cortisol, test anxiety appeared to also influence recovery in sAA following the stressor, with low test-anxious individuals who received positive training showed a blunted recovery relative to low anxious individuals who completed sham training.

These findings allude to suggestions that prior anxiety might mediate the relationship between bias and the stress response and, consequently, might also determine suitability of CBM training.

Study six. In two experiments, Study 6 tested the immediate robustness of trained attentional (dot probe training; Study 6a) and interpretive (homograph training; Study 6b) biases. Both experiments shared the same experimental design. Participants completed a single session of positive training (CBM-A or CBM-I) before completing an "untraining" session, which was composed in exactly the same format but without the training contingency. For example, in CBM-A training, the probe was consistently placed behind the positive word whereas probes in the untraining session were placed behind positive or negative words with equal frequency. Alternatively, CBM-I training consisted of constantly drawing on the neutral interpretation of the homograph, whereas untraining drew both on neutral and negative meanings with equal frequency.

Following an unexpected initial absence of training effects in Study 6a, and in consideration of recently published findings, participants were allocated into retrospective groups according to a median split of the sample's baseline bias measures. Following this, a significant interaction between group and bias was identified. Training was found to be effective in participants who were allocated to the negative bias group (i.e. those with a baseline bias that was lower than the median score). Further, in this sub-sample, the process of untraining was found to bear no influence on these improvements. Alternatively, participants allocated to the positive bias group (i.e. those with a baseline bias score higher than the median) showed no effects of training or untraining. In an attempt to directly compare the effects of CBM training between studies, participants in Study 6b were retrospectively allocated into positive and negative starting bias groups in the same fashion. This revealed exactly the same findings, with participants in the negative starting bias group

showing significant training effects that survived untraining while no training effects were present in the positive starting bias group. Further, in this latter sub-sample, biases were found to become significantly more *negative* following the untraining session. This study is the first to demonstrate negative effects of a positive CBM training, and suggests that the techniques might be suited only to people who might reasonably benefit from them.

9.2 Physiological Responses to Stressor Tasks

Following initial analysis, none of the stressor tasks employed in the studies presented here (Studies 1, 4, and 5) appeared to successfully elicit a physiological stress response. While Study 1 additionally showed no evidence of changes on standardised stress questionnaires, Studies 4 and 5 did induce feelings of rejection (Study 4), as well as stress, anxiety, and negative affect (Studies 4 and 5). Even with these significant psychological changes, certainly in Study 5 there appeared to be a distinct absence of any clear shifts in cortisol and sAA. To explore reasons behind these apparent contradictions, task selection and timings of sample collections will be discussed in turn.

9.2.1 Task selection. ***Study one.*** The lack of a response both on a psychological and physiological scale following the social rejection task employed in Study 1 brings about the conclusion that the task per se may have been at fault. In spite of the care taken to research appropriate stressor tasks, there are several grounds that, with hindsight, are argued to significantly contribute to the overall unsuccessful employment of this task (see *Study 1: Discussion*). As an example, the delivery of the stressful aspect of this task involved the researcher informing the participant that they had not been selected by any of their peers for the group exercise. This entire discourse, including the researcher entering the room, providing the information, and setting the participant up on the group task alone, took no longer than a couple of minutes. The purpose of subsequently actually completing the group

exercise (alone) was two-fold, both to enable participants time to ruminate on their rejection, which has been found to intensify cortisol reactivity (Zoccola, Dickerson, & Zaldivar, 2008), and to follow through on the original study brief. It is possible, instead, that the information was received and the group exercise then provided a useful distraction from the participant's brief embarrassment of being rejected by the group. In sum, while the task contained both elements of a socio-evaluative and uncontrollable nature, it did so with insufficient intensity and consequently was unsuccessful in acting as an acute stressor.

Study five. Study 5 was successful in significantly eliciting a psychological stress response, yet apparently did not stimulate any significant physiological response. In consideration of the fact that this task employed all aspects of Dickerson and Kemeny's (2004) three key factors it is likely that, in this circumstance, alternative reasons underlie the absence of changes in sAA (see *Sample collection points* below). Alternatively, there is an argument to suggest that cortisol did respond to the acute stressor task.

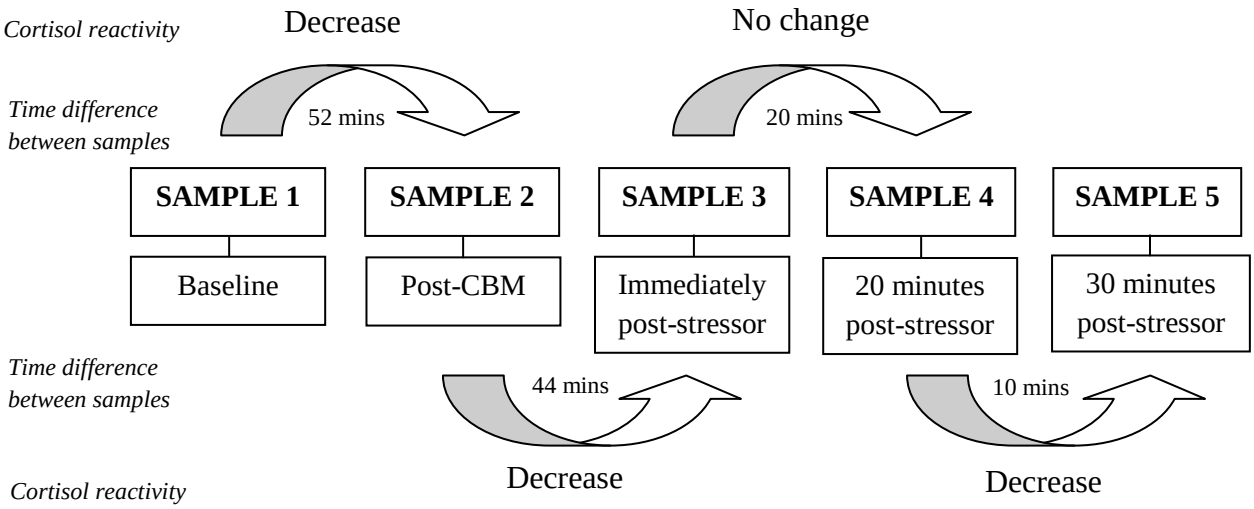

Figure 30. A flow chart showing the structure of samples and cortisol reactivity throughout Study 5.

As shown in Figure 30, cortisol was found to decrease generally across the study with the exception of between samples 3 and 4, which represented the 20 minutes following the end of the stressor. Adopting Blackhart, Eckel, and Tice's (2007) justification, this pattern could be interpreted as cortisol's natural diurnal decline being interrupted through external activation. This would imply that, by cortisol holding constant, individuals were actually displaying a HPA response. However, this interpretation must be drawn with caution as there is no control comparison group that did not complete the stressor task therefore this must remain only a possible interpretation and future studies would be necessary to test this possibility further. Furthermore, if authentic, the effects appear quite transitory, as a decrease in cortisol is evident just 10 minutes later (at sample 5). Though speculative, this inference is supported by the changes in psychological state (e.g. reported stress, etc.) and would further support the argued masked sympathetic (sAA) effects of the task (see *Sample collection points* below).

Study four. Arguably the task used to induce stress in Study 4 did induce a significant sympathetic response albeit not as intended. A regressional design was adopted to analyse the data in Study 4, to most appropriately address the main research question regarding the predictive capacities of natural attentional and interpretive bias. For this reason, ANOVAs were not conducted to directly assess the physiological impact of the social ostracism task alone. Addressing that topic retrospectively, a 2 (condition: social ostracism, social inclusion) x 4 (time point: baseline 2, post-OCam 1, post-OCam 2, and 30 minutes post-OCam 2) repeated measures ANOVA reveals a significant change in sAA secretion over time, $F(3, 201) = 11.42$, $p < .001$, $\eta_p^2 = .15$, that is not qualified by any significant interaction by condition, $F(3, 201) = 1.13$, $p = .34$, $\eta_p^2 = .02$. Though this result is unexpected (specifically, finding a main effect that is *not* qualified by a significant interaction), further investigation demonstrates a significant increase in sAA only following the first (neutral) OCam, $t(74) = -$

5.30, $p < .001$, $d = .50$. At this stage, participants have experienced exactly the same study protocol, hence the absence of any interaction by condition is understandable. There is no significant change in sAA between the two OCam videos (the second of which contained the social manipulative element), $t(72) = 1.60$, $p = .12$, $d = .16$, however following OCam 2 there is a significant drop in sAA, $t(71) = 2.83$, $p = .01$, $d = .28$ (see Figure 31).

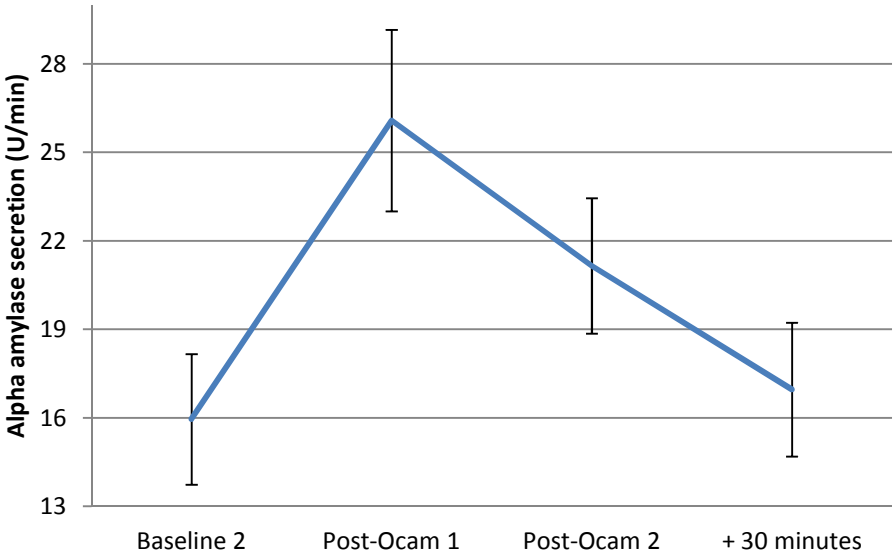

Figure 31. sAA reactivity over Study 4.

The above findings imply that, while public negative evaluation failed to activate the sympathetic stress response, the act of self-presentation (i.e. merely participating in the OCam video tasks) was successful in doing so. Perhaps this finding further indicates that, while acute stressor tasks are advised to contain the opportunity for social evaluation, this is necessary only as a potential outcome rather than an actual one. This speculation is supported by Brosschot, Gerin, and Thayer's (2006) perseveration cognition hypothesis, in which a large degree of physiological activation is argued to be due to thinking about stress. Put another way, and drawing on a famous quote from Shakespeare, "There is nothing either good or bad, but thinking makes it so".

Turning to the cortisol response, a 2 (condition: social ostracism, social inclusion) x 4 (time point: baseline 2, post-OCam 1, post-OCam 2, and 20 minutes post-OCam 2) repeated measures ANOVA also showed a significant main effect of time, $F(2.27, 106.45) = 5.41$, $p < .01$, $\eta_p^2 = .10$ (Greenhouse Geisser corrected), with no significant interaction by condition, $F(2.27, 106.45) = .35$, $p = .73$, $\eta_p^2 = .01$. Explored further, cortisol was found to remain unchanged between samples 2-3 (baseline 2 – post-OCam 1), $t(59) = 1.37$, $p = .18$, $d = .10$, and 3-4 (post-OCam 1 – post OCam 2), $t(54) = -1.01$, $p = .32$, $d = .05$, before showing a significant decrease between samples 4-5 (post OCam 2 – 20 minutes after post OCam 2), $t(55) = 3.74$, $p < .001$, $d = .21$. Recalling the finding of a significant decrease in cortisol between samples 1 (baseline 1) and 2 (baseline 2), this finding appears to corroborate the suggestion that the act of social presentation acted as a sole acute stressor. Again drawing on cortisol's natural decline throughout the day (e.g. Buchanan, Kern, Allen, Tranel, & Kirschbaum, 2004), the noted decreases in cortisol between samples 1-2 and 4-5 could indicate an uninterrupted natural rhythm (see Figure 32). During the 20 minutes between samples 2-3, and the 10 minutes between samples 3-4 (30 minutes in total), cortisol levels remain unchanged. During this time, participants were preparing for and taking part in the self-presentation tasks. Arguably, this could again be interpreted as a disturbance of the diurnal rhythm caused by exogenous activation of the HPA axis. As with sAA patterns of response the evidence suggests that, while actual social rejection was unsuccessful in inducing any physiological activation, the mere possibility of socio-evaluation embedded in the act of self-presentation succeeded in doing so.

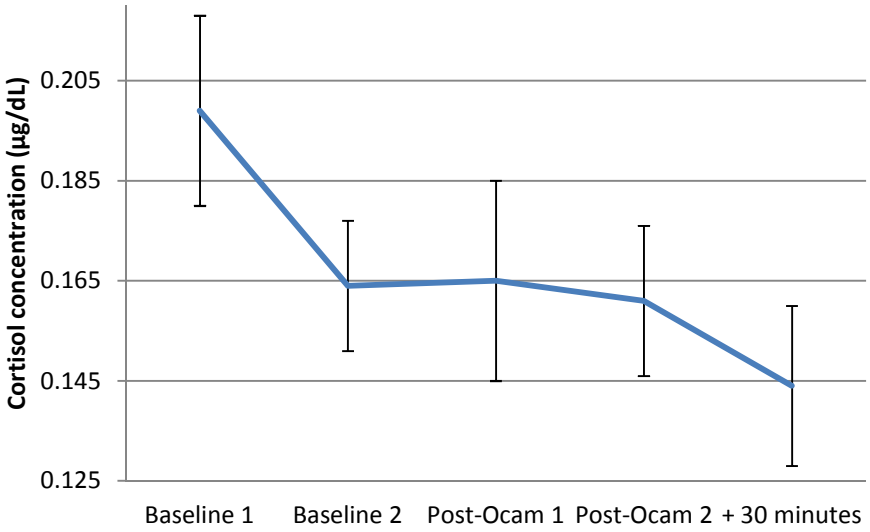

Figure 32. Cortisol reactivity throughout Study 4.

9.2.2 Sample collection points. As sAA is released almost instantly following neural and cellular changes (see Nater & Rohleder, 2009, for a review), changes in response to an acute stressor would be expected imminently. Alternatively, cortisol is the end-product of a cascade of hormonal changes and therefore, with the transfer time from serum into saliva, stress-induced changes are normally subject to a 10-20 minute post-stressor delay (Kirschbaum & Hellhammer, 1989). While researchers typically position the sample collection points in accordance with these considerations (see Figure 33 for an illustration of the designs used in the studies presented in this thesis), it is possible that mistiming collection points risks inadvertently missing peak changes. The likelihood of this occurring for sAA mounts as the time taken to complete the stressor task increases. However, in consideration of the difficulty in eliciting cortisol responses through laboratory stress procedures (Dickerson & Kemeny, 2004), researchers intending on measuring both ANS and HPA responses to a single challenge are presented with the dilemma of finely balancing optimal conditions to observe changes in both systems.

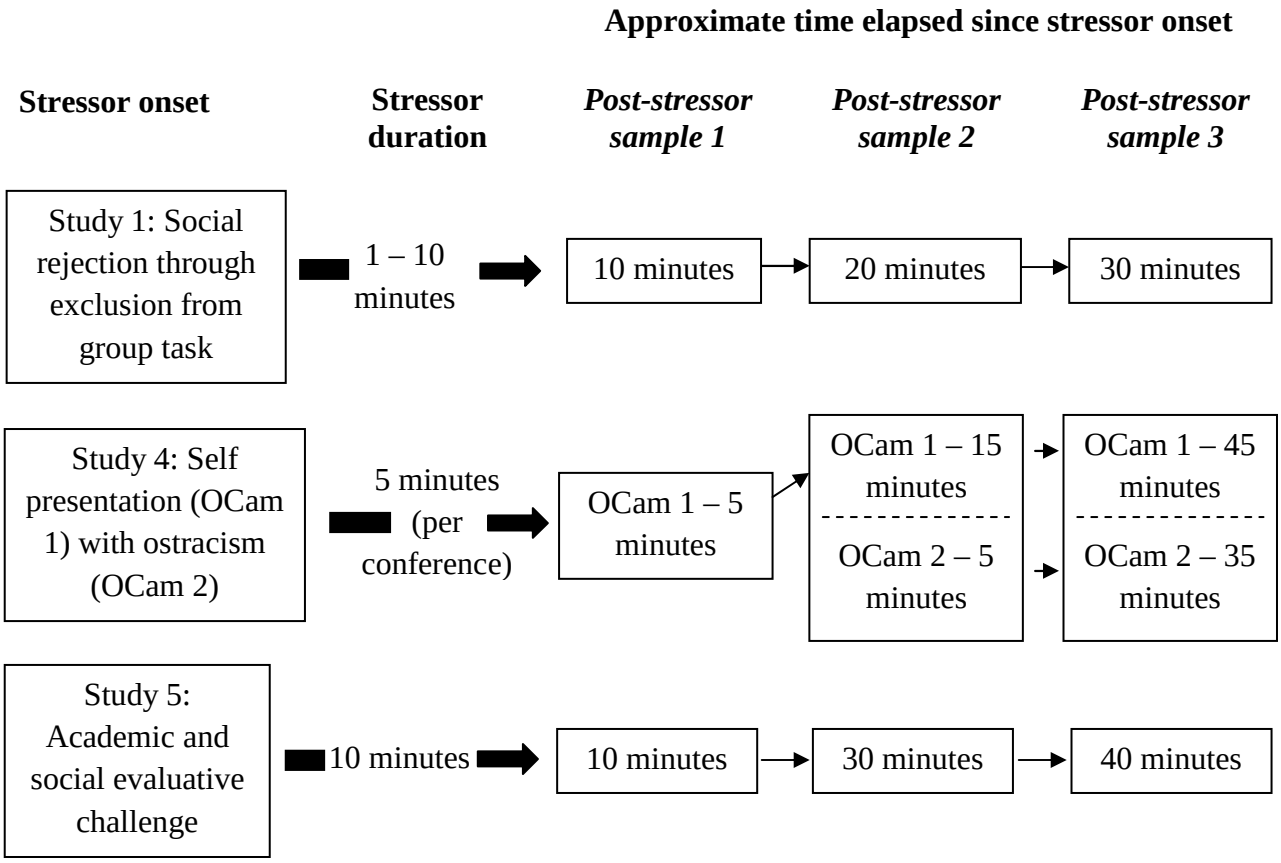

Figure 33. Flow chart to show stressor / saliva sample structure.

Certainly in Study 5, which consisted of a 10 minute stressor, it is likely that the sample taken immediately after the task failed to capture the peak sAA response due to the reasons discussed above. This is supported by the fact that the secretion rate was found to increase following the stressor, though was not found to be significant. As a further unintentional confound in documenting the stress-induced change in sAA in Study 5, secretion rate was found to increase between the first two samples of the study (sample 1: baseline; sample 2: post-CBM training; see Figure 22 below, reproduced from Study 5).

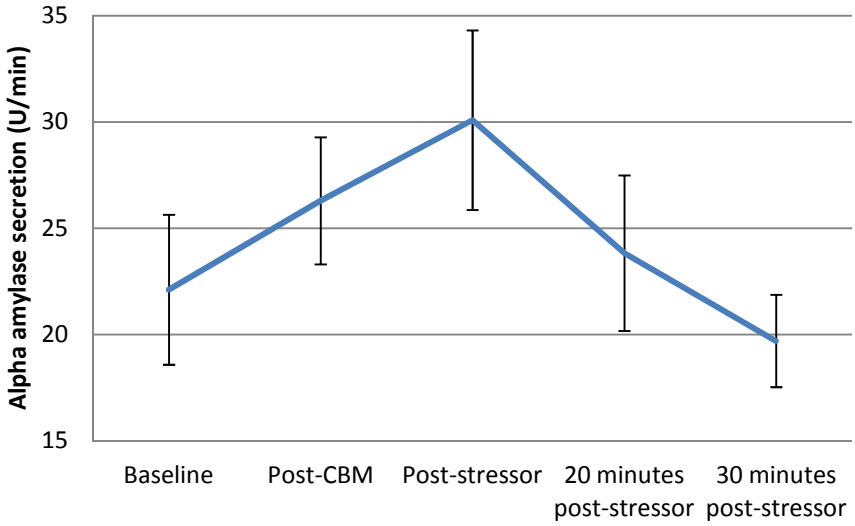

Figure 22. Changes in alpha amylase secretion over the study (collapsed across conditions). Reproduced from Study 5.

The increase in sAA evidenced between the first two samples presents two problems as, firstly, the ensuing (non-significant) increase between samples 2-3 could be attributed either to participation in the stressor or could have carried over from the previous cause. Second, it removes the existence of a reliable pre-stress measure of sAA. As sample 1 (baseline) was taken after a practice sample, at approximately 25-30 minutes into the study, it is arguably an accurate and reliable baseline measure. However, the rise in sAA documented between the first two samples rules out the use of sample 1 as an appropriate baseline against which to compare stress-related changes in sAA. Alternatively, sample 2 is not an ideal pre-stress measure either as it now contains the inherent inflation in sAA. With hindsight it is possible to argue that these factors probably substantially contributed to not finding a significant increase in sAA following the stressor.

Summary. Though none of the tasks employed in Studies 1, 4, or 5 were successful in eliciting an *increase* in cortisol, arguments presented above indicate that Studies 4 and 5 may have managed to activate the physiological stress response systems in the desired manner to some extent. As is evident, using salivary biomarkers to measure physiological

changes to acute stressors presents a challenge in itself. Where possible, future studies using salivary biomarkers are recommended to adopt a simple design where participants are not required to complete several different tasks sequentially on the same day. In Study 5, this was proposed to interfere with accurately documenting stress-related changes in sAA. Further, sample collection points need to be carefully positioned in the design of the study to prevent peak reactivity being missed, which is not always an easy feat (e.g. Study 1).

Though the tasks used in Studies 4 and 5 appear to have elicited activation of the physiological stress response systems to a certain degree, they are clearly not as effective as some of the more established procedures. For example, the Trier Social Stress Test (Kirschbaum, Pirke, & Hellhammer, 1993) appears to be the most reliable of the popular choices of acute stressors due to its apparent capacity to consistently evoke increases in cortisol regardless of typically confounding factors such as diurnal variation (e.g. Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004). In their meta-analysis, Dickerson and Kemeny (2004) argue that this is due to the task including elements of uncontrollability, socio-evaluation, and being personally relevant. However, the results from Study 4 suggest that the perception of these factors might be more essential than their actual occurrence. Participation in the self-presentation aspect of the OCam paradigm was sufficient to elicit increases in sAA and cortisol, while actual ostracism only served to worsen reported psychological states and had no effect on physiological responses. To an extent, the actual rejection aspect could be argued to have reduced the impact of uncontrollability, as it forces the move from a “What happens if they don’t like me?” to a “They don’t like me, how will I cope?” mentality.

Compared with more continuous methods of measuring physiological response, such as continuous blood sampling to capture HPA activation or heart rate or electrodermal tracking to monitor ANS activation, saliva does provide a practical and minimally invasive

alternative. However, this thesis has documented some problems associated with collecting accurate and reliable samples. Through applying the recommendations noted in Studies 2 and 3, specifically using secretion rate calculations of sAA for the purposes of analyses, consistent implementation of a practice sample, and collecting samples with the aid of a swab, no further problems were encountered in Studies 4 and 5. Therefore, with such cautions heeded, saliva is still recommended to be the most suitable option for investigations into biobehavioural stress research.

At this point it is noteworthy to consider the reasons for employing stressor tasks in terms of the scope of this thesis. The primary aim of this thesis was to investigate the relationship between cognitive biases (natural or modified) and the psychophysiological stress response. Of the limited range of studies that also address this subject using similar strategies, not all have observed significant increases in stress biomarkers following a "stress task" (e.g. Hoppitt, Mackintosh, Randall, & Bristow, under review). Nevertheless, interesting results have been noted in spite of this absence suggesting that the much anticipated main effects of task are not necessary in such investigations. Even so, the absences of main effects might explain why no influences of bias were evident at the initial response stage. Future research might seek to investigate this outstanding question using the TSST which, though expensive and time-consuming to conduct, has been commonly shown to elicit cortisol responses.

9.3 Bias and the Stress Response

Study 4 measured the predictive capacity of interpretive and attentional cognitive biases on the psychological and physiological response to an acute stressor, while Study 5 explored the influence of a single session of CBM-I on the psychological and biological effects of acute stress. Whereas previous studies have shown evidence of more positive biases

(either natural or through bias modification training) being consistently associated with a reduced psychological impact of an acute stressor (see Beard, 2011, for a review), the studies presented within this thesis found only limited support for this link. Further, initial interpretations of the findings from the research presented in this thesis demonstrated a lack of generalisation of bias influences in terms of physiological reactivity to stress. However, further consideration of similar literature reveals justification for some findings and exciting innovations for others.

9.3.1 Attentional bias. In Study 4 attentional bias was found to serve as an independent predictor of changes in negative affect in response to participation on the first OCam task. This task was found to act as a stressor, with significant increases in reported negative affect and stress. However, attentional bias was not found to significantly predict changes in reported stress or positive affect during this time, nor was it found to predict changes in any of these variables following the second OCam task. These findings therefore provide only limited support for previous research that demonstrates a robust link between biased attentional processes and psychological vulnerability to stress. Of further interest, attentional bias was not found to significantly predict changes in sAA or cortisol during this time. As evidence presented in earlier sections of this discussion suggests, both measures of physiological activity are argued to have responded to the self-presentation aspect of the OCam. Therefore the data suggests that attention biases do not significantly influence an individual's physiological response to stress.

These findings are partially supported by Fox, Cahill, and Zougkou (2010), who found pre-existing attentional biases to be predictive of subsequent cortisol response to acute stressors presented either 4 or 8 months later only when measures of attentional bias included masked stimuli. Masked stimuli were presented on screen for just 14 milliseconds, and therefore remained outside the bracket of conscious awareness. Alternatively, stimuli that

were unmasked (300 millisecond presentation period) were not found to significantly predict cortisol responses to either acute stressors. Fox et al. argue that their findings demonstrate evidence that early stage (i.e. preconscious) processing biases are more influential in predisposing vulnerability to anxieties relative to later stage conscious biases. As Study 4 included only unmasked stimuli, the absence of any predictive powers of attentional bias could be seen as support for Fox et al.'s supposition.

Koster, Baert, Bockstaele, and De Raedt (2010) explored the potential for CBM-A training (dot-probe) to influence early (unconscious) and late (conscious) stages of processing biases. Participants were found to show no effects of training when training stimuli were masked, with presentation controlled at either 30 milliseconds or 100 milliseconds. When stimuli were unmasked (500 millisecond presentation), participants showed the typical changes in bias following training, with a reduced attention bias to threatening materials following positive training but not control (sham) training. This replicated findings by MacLeod, Rutherford, Campbell, Ebsworthy, and Holker (2002; Experiment 1), who also found CBM-A to be ineffective when stimuli were presented outside of conscious awareness (20 milliseconds).

While proving effective in attentional bias modification, under Fox et al.'s notion (with support from Study 4 findings) that only early (preconscious) attentional biases are influential in predicting individuals' physiological vulnerability to acute stress, one might expect typical attentional bias modification procedures (that work within conscious awareness) to be ineffective in reducing the physiological impact of stressors. However, in the only published study to date of its kind, Dandeneau, Baldwin, Baccus, Sakellaropoulo, and Pruessner (2007) do identify a potential for unmasked (and therefore conscious) attentional bias modification to affect physiological stress. Using a slightly different method to the conventional dot-probe task, participants were required to complete trials in which they

located a still image of a face showing a neutral expression amongst a 4x4 matrix of photos of people looking angry. Participants were a group of telemarketers, who experience a high degree of occupational stress associated with making cold calls, such as continuous rejection. Following a five day training period, participants who completed this find-the-happy-face (as opposed to a find-the-flower control task) released significantly less cortisol over the final working day (the assumed stressful event). Despite the authors referring to their training as targeting early stages of attention, the method forces participants to make a conscious decision and so would likely be classified by both Fox et al. (2010) and Koster et al. (2010) as being directed towards later stages of attention. As such, and according to Fox et al.'s hypothesis, the training should not have been effective in reducing physiological activation. However, it could be argued that Dandeneau et al.'s training technique operated by encouraging a rapid conscious disengagement from threat cues, as participants were required to find the single neutral facial expression amongst the remaining 15 unhappy facial expressions. Therefore, with the source of stress associated with working as a telemarketer originating from the potential for rejection resulting from making cold-calls, this type of training seems perfectly tailored for such a sample group for two reasons. In addition to promoting active disengagement from such rejection, the training might act almost as a fixed reinforcement schedule as participants would learn that eventually they would always find the neutral face in the 4x4 matrix. This could arguably transfer onto an individual's appraisal of cold-call success and amend their method of coping with rejection.

9.3.2 Interpretive bias. In Study 4, interpretive bias emerged as a significant independent predictor of changes in reported stress following participation on the self-presentation (OCam 1) task, though not of changes in reported positive or negative affect. Again, this only partially supports existing literature that claims a link between biased cognitions and stress vulnerability. No finding emerged suggesting interpretive biases

influenced changes in sAA or cortisol during this time. Interestingly, however, interpretive bias did emerge as a trend predictor of sAA change between the final two samples. As discussed earlier, during this time a significant reduction in sAA secretion was found that indicates a recovery period from the acutely stressful experience of self-presentation. This finding therefore hints that, while attentional biases might be associated with initial reactivity to a stressor, interpretive biases might alternatively influence how fast individuals recover from acute periods of stress.

Study 5 then investigated the effects of a single session of CBM-I (vignettes-based training) on the psychological and biological response to an acute stressor. While a decrease in cortisol was found following the CBM-I training, this has since been argued to be resulting from the natural diurnal decline in cortisol (e.g. Buchanan, Kern, Allen, Tranel, & Kirschbaum, 2004). Training was not found to influence individuals' physiological responses to the stressor, though participants who completed sham training relative to a positive training programme had trend higher levels of cortisol and significantly greater amounts of reported stress throughout the study. This suggests that positive CBM-I training might have some general soothing effects on stress activation. Further, some interesting patterns emerged that again indicated an influence of interpretive bias on recovery from stress. Of note, this pattern additionally depended on subjective test anxiety (recalling the relevant fact that the stressor task was test-based). Following a median split, participants low in test anxiety exhibited no change in cortisol between samples 4-5, while participants high in test anxiety showed a significant decrease in cortisol following positive CBM-I training but not following sham training. Further, while there was no change in sAA secretion between these time points in individuals high in test anxiety, low test anxious individuals showed a significant increase in sAA secretion following positive training but no change following sham training. Though

initially perplexing, the findings of Study 6 goes some way to clarifying reasons behind these responses.

Participants in Study 6 with a stronger negative interpretive bias (following a median split of entering bias scores) were found to respond in the expected manner to a single session of positively valenced homograph-based CBM-I training; training was effective, which was evidenced by a significant improvement in bias. Alternatively, participants with a stronger positive bias were found to show no effects of training, with bias scores remaining unchanged. Further, following repeated exposure to training material without any probe/valence contingency (a phase of the study designed to test the robustness of freshly trained biases), participants who had started with a more positive bias showed a decline (becoming more negative) in bias scores. In contrast, the bias of participants who had benefitted from training (those starting with a relative negative bias) appeared unaffected by this untraining period. Applying these findings to the patterns of response in Study 5 highlights some interesting and clinically relevant points.

In consideration of the strong link between anxiety and interpretive bias (e.g. Beard & Amir, 2009), it is plausible to assume that participants in Study 5 who scored highly on measures of test anxiety might also possess a tendency to disproportionately interpret ambiguity in a negative manner (i.e. have a negative interpretive bias). In light of the findings of Study 6 that imply that CBM procedures might only be suitable for participants who would directly benefit from them (i.e. those with a negative bias), the observed interactions between test anxiety and physiological activation appear more logical. Participants higher in test anxiety (and so arguably with stronger negatively biased interpretations of ambiguity) showed an improved recovery to stress when they received positive CBM-I training relative to sham CBM-I training. Alternatively, participants lower in test anxiety (arguably those with a stronger natural positive interpretive bias) recovered better from acute stress when they

received *sham* training and, further, showed evidence of a poorer recovery time following positive training.

9.4 Clinical Potential of CBM Methods

Although not directly exploring the relationship between bias and the psychophysiological stress response in clinical samples, the present findings can be applied to the clinical potential of the development of CBM treatment tools. Many clinical disorders are characterised by hyper-arousal and hyper-vigilance to threat. For example, a predominant feature of generalised anxiety disorder (GAD) is an excessive tendency to worry (e.g. Gosselin & Laberge, 2003). In physiological terms, the chronic stress associated with such propensities is linked with individuals being less able to exhibit a sympathetic response to episodes of acute stress (Fisher, Granger, & Newman, 2010). Further, Fisher et al. found that higher baseline levels of sAA in participants with GAD were predictive of a smaller sympathetic reactivity relative to lower baseline levels. Taken together, these findings indicate a general dysregulation of ANS activity that is characterised by a chronic hyperactivity leading to reduced reactivity potential.

While experimental research has demonstrated a clinical potential in treating GAD by retraining attentional biases (e.g. Amir, Beard, Burns, & Bomyea, 2009), these investigations have so far focused only on (conscious) attentional biases and have also not yet considered the effects of any associated physiological activation. Results from Studies 4 and 5 indicate a potential for interpretive biases to moderate subjective recovery from stress. If authentic, these findings would significantly advance the potential for CBM-I procedures in a clinical setting. For example, effective recovery following episodes of stress might serve to break the chronic hyper-activity cycle associated with anxiety disorders and restore the reactivity potential much in the same way that relieving pressure from an elastic band restores its

capacity to stretch again in the future. This might further reduce the risk of stress-related physiological ailments linked with chronic hyper-arousal, such as a build up of atherosclerotic plaque in the arteries caused by excess cortisol (e.g. Dekker et al., 2008) that can significantly elevate the risk of cardiovascular disease.

Of additional significance in applying CBM techniques to a clinical research setting, the findings of Studies 5 and 6 imply that CBM methods are not suitable for all populations. For those suitable to the techniques, the effects appear robust following exposure to immediate efforts to out-train it. Alternatively, participants who logically would not have benefitted from positively-valenced CBM training appeared in some instances to respond adversely to its forced exposure. This second point is more relevant to the development of CBM techniques only where studies employ control comparison groups, to uphold ethical responsibilities linked with protecting the well-being of research participants.

9.5 Limitations and Future Research

9.5.1 Stages of attention. The research presented and discussed here suggests that there remain some inconsistencies in the documented literature investigating the relationship between biased cognitions and the psychophysiological stress response. For example, evidence suggests that attentional biases are only capable of predicting physiological responses to acute stressors when they exist outside of an individual's conscious awareness (Fox, Cahill, & Zougkou, 2010). As most attentional bias modification procedures operate at a level within conscious awareness, arguably these methods would be ineffective in modifying an individual's physiological vulnerability to acute stress. However, Dandeneau, Baldwin, Baccus, Sakallarpoulo, and Pruessner (2007) have demonstrated the beneficial effects of such procedures thereby directly contradicting the assumptions of Fox et al.. Further, while no studies exist that investigate the effects of masked attentional bias

modification procedures on the physiological stress response, research has shown it to be ineffective at modifying attentional biases and psychological responses using this method (Koster, Baert, Bockstaele, & De Raedt, 2010; MacLeod, Rutherford, Campbell, Ebsworthy, & Holker, 2002). Clearly a deeper understanding is needed to clarify such discrepancies, which might further illuminate reasons behind the inconsistent successes of CBM methods in different clinical samples.

Currently it is understood that highly anxious individuals display a vigilance-avoidance pattern towards threat, which is characterised by a disproportionate attention towards threat during the early stages of information processing followed by threat-avoidance in the later stages (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; Koster, Verschuere, Crombez, & Van Damme, 2005). Most studies using dot-probe modification tasks claim that the procedures target early information processing stages to retrain the propensity to automatically focus attention on threatening stimuli. If true, the basis by which training works is entirely understandable. Indeed, the methods have been shown in a number of cases to reduce clinical levels of anxiety (e.g. Amir et al., 2009; Schmidt, Richey, Bucker, & Timpano, 2009). However, Koster et al. (2010) claim this not to be the case, instead proposing that typical attention modification methods work on later stages of attention. Further, Koster et al. suggest this to be a contributing factor in explaining why the potential of CBM has not been as prominent in reducing phobia-specific anxieties. According to fear theorists (e.g. Foa & Kozak, 1986), phobic participants need to consciously attend to the phobia-related stimuli in order to habituate to it. Koster et al. claim that using typical (in their opinion late stage) attentional training methods therefore only serves to reaffirm their existing patterns of response which maintains their fear and, consequently, their phobia fails to improve.

Alternatively, it is possible that typical attentional bias modification procedures do in fact target early (though not preconscious) stages of attention. That is to say, the stimuli are presented within the bracket of conscious awareness, but the association between trained contingencies remains implicit. Assuming this is true, the reasons behind the less evidenced beneficial effects of CBM in phobias could be attributed to the constructs that underlie these more specific fears. For example, Van Bockstaele et al. (2011) argue that CBM methods are more suitable to disorders that are featured by broader rather than specific threats. Phobias are associated with significantly larger physiological responses upon exposure to related threats relative to individuals suffering from other clinical anxiety disorders, such as SAD or GAD (Lang & McTeague, 2009). Van Bockstaele et al. therefore propose that CBM methods successfully retrain anxieties but, in their current format, will remain ineffective in retraining more specific fears.

Clearly considerably more exploration of the topic is necessary before the principles that underpin CBM-A are fully understood. Specifically, it would be useful to understand which stages of attention (preconscious, early, or late) are currently targeted by CBM-A, in addition to discovering which stages might best be targeted to promote lasting positive changes.

9.5.2 Inconsistent findings. In Study 4, interpretive bias was found only to predict changes in self-reported stress following self-presentation, while attentional bias was found only to predict changes in reported negative affect during this time. On initial interpretation, this appears to largely contradict the general consensus of published research that demonstrates a clear and robust link between cognitive biases and psychological stress vulnerability. However, generally both measures of bias have previously been compared against reported anxiety in prior literature (e.g. Fox, Cahill, & Zougkou, 2010; MacLeod, Rutherford, Campbell, Ebsworthy, & Holker, 2002; Wilson, MacLeod, Mathews, &

Rutherford, 2006). Therefore, a possible alternative explanation drawn from the research presented here might suggest that, while both measures influence the overall perception of stress, they work on specific aspects of the multifaceted process. Naturally, future research might seek to explore this further. If this alternative hypothesis is upheld, a future goal of future CBM research might consequently be to develop tailored treatment tools according to specific subjective needs.

While Study 5 found no influence of CBM-I on psychological experience following an acute stressor, direct comparison between study designs adopted here and those used in previous studies might clarify potential reasons for the observed discrepancies. Early studies that supposedly demonstrate the potential of CBM on acute stress vulnerability could be criticised for their choice of "stressor" tasks used. For instance, while unpleasant, watching footage of an accident would arguably not lead to an increased state of stress in the majority of people. Indeed, in two studies that used such a task, one found no main effect of time (pre- vs. post-task) on reported anxiety (Mackintosh, Mathews, Yiend, Ridgeway, & Cook, 2006) while the other found only a trend effect ($p < .10$; Wilson, MacLeod, Mathews, & Rutherford, 2006). Alternatively, the task employed in Study 5 led to clear and significant increases in reported stress, anxiety, and negative affect (all p values < .001; all effect sizes > .64). It is possible, therefore, that the earlier tasks brought about more subtle changes in psychological state, which a single session of CBM was more able to influence. Further, where studies have more recently demonstrated the potential of CBM either in clinical samples (Amir et al., 2009; Beard, Weisberg, & Amir, 2011; Brosan, Hoppitt, Shelfer, Sillence, & Mackintosh, 2011; Schmidt, Richey, Buckner, & Timpano, 2009) or outside of the laboratory using real-life stressors (Dandeneau, Baldwin, Pruessner, Baccus, & Sakellaropoulo, 2007; See, MacLeod, & Bridle, 2009), they have typically done so using multiple sessions of CBM training. For this reason, relationships between psychological

vulnerability to stress and CBM (as well as stronger relationships with physiological stress) in Study 5 might have emerged following a more rigorous CBM training schedule.

9.5.3 Stigmatising the stress response. It appears to be assumed that in order for CBM methods to be considered effective, they would lead to a reduced physiological response to acute stressors. However, just as authors involved in the early investigations into the relationship between cognitive bias and anxiety vulnerability cautioned readers not to assume causality, so too should we take necessary precautions in labelling "good" and "bad" stress responses. As discussed at the beginning of this thesis the physiological stress response is, in its most basic form, designed to aid the body in times of stress. Recent research has shown that exogenous cortisol administration serves to *decrease* preconscious attention to threat (Putman, Hermans, Koppeschaar, can Schijndel, & van Honk, 2007; Putman, Hermans, & van Honk, 2010), which illustrates a certain acute anxiolytic-like effect of cortisol. Where the stress system operates on a normal level, it makes sense that acute increases in cortisol serve to redirect attention from threat to enable the organism to fight or flight, rather than stand rigid with fear.

Where the stress response system operates with a dysfunctional rhythm, this finding might also account for how clinical anxiety disorders are maintained. As previously noted, individual's suffering from anxiety disorders often display a blunted physiological reactivity potential (e.g. Fisher, Granger, & Newman, 2010). Assuming that cortisol does exert some transient protective properties over attention to threatening stimuli, the absence of an acute physiological response might therefore serve to prolong attention to threat rather than reduce it. Consequently, in this instance, CBM techniques might serve as an effective strategy to realign biased cognitions and restore healthy levels of physiological activation. Needless to say, such a hypothesis would need considerable further investigation, though brings to light the potentially complex relationship between emotive, cognitive, and physiological defences.

9.5.4 Response or recovery? In the literature that investigates the effects of CBM on anxiety and stress vulnerability, it is typical for researchers to solely classify stress vulnerability in terms of how an individual *responds* to a stressful event. For example, See, MacLeod, and Bridle (2009) implemented a home-based attention training programme involving 15 days of training prior to individuals relocating to an alternate country to study (stressful life event). State anxiety was measured prior to the training and on the move day, with results indicating that participants who had received positive training (relative to a control programme) reported significantly smaller increases in anxiety arising from the transition. The structure of this study design is typical in that researchers include a baseline measure of stress (or anxiety, etc.) and measure immediate responses to a stressor, but fail to include any follow-up measures indicating how efficiently individuals recover from these stressful events. Even in studies measuring responses to more acute laboratory stressors, there is a notable lack of focus on recovery (e.g. MacLeod, Rutherford, Campbell, Ebsworthy, & Holker, 2002; Salemink, van den Hout, & Kindt (2009).

In Studies 4 and 5, interpretive bias (either natural or trained) appeared to exert some influence on an individual's capacity to recover from acute episodes of stress. Stronger positive biases were associated with a faster physiological recovery to stress-related activation relative to stronger negative biases. While studies exist that compare the psychological responses to acute stressors with interpretive biases, the present research is original in its inclusion of physiological measures of stress. Therefore, while not reproducing findings to show CBM-I training leading to attenuated psychological responses to stressors (Study 5), the research does substantially contribute to the current literature by offering an explanation into how biased cognitions might operate synergistically to disrupt internal harmonisation.

Just as Lazarus (1991) identified two stages of appraisal, so too could there exist two similar stages of interpretive bias; one involved with the initial judgement of threat, and a second involved with the coping potential and recovery from stress activation. Both stages of interpretive bias could function together with attentional biases to dictate an individual's overall response to threat. For example, with attentional biases governing how frequently threat is detected and primary stages of interpretive biases determining the extent of an individual's initial response (as noted already in the literature), secondary stages of interpretive bias could then be involved in an individual's recovery success (as noted in Studies 4 and 5). Participants with stronger positive secondary biases might reappraise the threat, and commence the process of recovery, while individuals with stronger negative biases might ruminate on the stress or their response to it, thereby extending its effect and delaying recovery. This theory ties in with the Perseverative Cognition Hypothesis (Brosschot, Gerin, & Thayer, 2006), which assumes that "thinking about" or lingering on stress can serve to prolong its impact through anticipatory effects and delayed recovery. This extended response, if repeated regularly, could seriously disrupt homeostatic balance by overloading a system that was originally designed to provide an immediate but temporary solution. In other words, the stress response tap is being left turned on. Further tentative evidence for this might be taken from the finding that participants who received positive CBM-I training were found to release significantly less cortisol over the entirety of Study 5 relative to participants receiving sham training.

Assuming that interpretive bias does contribute to this prolonged activation there is the potential for CBM-I methods, which have to date largely been sidelined in clinical investigations for CBM-A targeted treatment, to greatly assist in rebalancing these overloaded systems. Moreover, a combination treatment that targets both an overactive inclination towards threat in addition to a healthier recovery from stressful episodes might

provide an even stronger treatment tool. Two recent studies have provided the first insight into this collective approach, with results demonstrating its success in clinical settings (Beard, Weisberg, & Amir, 2011; Brosan, Hoppitt, Shelfer, Sillence, & Mackintosh, 2011). The current results support the use of CBM-I methods, either alone or integrated with CBM-A training, and further encourage consideration of recovery abilities as a worthwhile aim of these treatment tools.

9.5.5 Control group. In the between-subjects design of Studies 1 and 4, efforts were made to include one experimental and one control condition. The social nature of the experimental condition in both studies (social rejection/ostracism) made it difficult to include a neutral reference condition, therefore both control conditions instead formed comparison conditions that were more reflective of non-rejection/non-ostracism (i.e. acceptance/inclusion). Blackhart, Eckel, and Tice's (2007) original design (which Study 1 was based on) did include a control condition, where participants were not assigned a group due to an administrative error rather than through being rejected or accepted. However, there was no significant difference between the control and the acceptance group in terms of positive or negative affect or cortisol responses to the task (Blackhart et al., 2007). For the purpose of Study 1, in light of this finding, an acceptance group was favoured over the administrative control group due to the procedure being more similar to the experimental condition (i.e. both received social feedback).

By using comparison conditions that encompassed acceptance/inclusion it is possible that fear of positive evaluation, which has been discussed in Studies 1 and 4 (*Discussion*), acted as a confounding factor. For this reason it must be recognised that, while the conclusions drawn from these studies remain valid in the contexts in which they were reached, the effects cannot exclusively be attributed to either one condition. Study 1 has since been concluded as unsuccessful in its overall aims, therefore this finding does not further

impact on any outcomes. For Study 4, the most interesting findings related to participants responding generally to the self-presentation aspect (a within-subjects factor) rather than the social manipulation (a between-subjects factor), therefore this point does not significantly detract from the original and interesting findings that have been revealed. Nevertheless, a design that includes an authentic control condition remains the ideal standard to provide substantial clarity in attributing intervention effects.

9.5.6 Single measure dependence. One potentially significant limitation of the studies contained within this thesis is in the reliance on solitary methods of measurement taken to represent key constructs. This was first observed in Study 1, where it was suggested that the method of saliva collection might be producing inaccurate and unreliable data. Studies 2 and 3 sought to overcome this in as timely as manner as possible without deviating from the main objectives of the thesis, and enabled the implementation of a best-practice methodology for Studies 4 and 5.

Alternatively, the methods used to measure interpretive and attentional bias and to train interpretive biases have not here been further investigated. It is important to note that that while the methods used were chosen for justifiable reasons, they are not without their limitations. In particular, the dot-probe task method of measuring biased attention has been criticised as having poor test-retest reliability in non-clinical populations. Schmukle (2005) explored this concept in a student population over a one-week period, with word pairs relating to physical (e.g. *tumour*) or social (e.g. *failure*) threat. Test-retest reliability across the week interval was not significant, while internal consistency was found to be very poor at first measurement for all word pair combinations ($\alpha = .00$) and at second measurement for social threat word pairs and for physical and social threat word pairs combined ($\alpha = .00$). For physical threat word pairs at the second measurement point, Cronbach's $\alpha = .06$. The finding of both internal inconsistency and unstable reliability led Schmukle to conclude that the dot-

probe task was an unreliable measure of bias index differentiation in this cohort. While this appears to support the unexpected absence of links between attentional bias and psychophysiological responses to stress in the non-clinical population in Study 4, that is not a reason to blindly accept the supposition. Indeed, certain limitations of Schmukle's methodology could account for the weak findings. For example, Cronbach's α was calculated by dividing each of the 64 trials into groups of 4 (16 groups) and computing an α score based on the ABI scores of these groups. Conventionally, ABI scores are derived from mean scores of all trials to account for factors such as target-probe placement, therefore it could be argued that splitting trials by group without controlling for these factors invites error and fabricated variability. Second, while Schmukle describes the sample as non-clinical, there is no descriptive data showing mean or range trait anxiety score of the group despite this being a factor known to influence attention bias (e.g. Mogg, Bradley, & Hallowell, 1994). Further, while studies that demonstrate the efficacy of CBM-A do suggest some stability of attention bias, it is possible that biases in attention might also incorporate a state-like element that is dependent on factors such as mood and alertness and environmental factors. If so, two measures of bias taken a week apart might not be expected to share a particularly strong correlation. Finally, while this point in itself is interesting, for the purpose of this thesis it only relates to Study 4 and arguably not the interesting patterns that emerged in Study 6 regarding sensitivity to CBM training. This is owing to the fact that Schmukle's study only investigated the reliability of the task as a bias measurement tool (i.e. without CBM training), consequently the effects cannot flippantly be generalised without further investigation.

9.5.7 Gender. As a noteworthy limitation in the external validity of this thesis, Studies 1, 4, and 5 only recruited female participants. Gender was controlled in this manner in recognition of the differing responses to acute stress procedures (e.g., Stroud, Salovey, & Epel, 2002) and in consideration of findings from Study 2, which suggested that females

might respond differently to the process of giving saliva samples for the purposes of research. These control measures were introduced in an attempt to reduce the amount of noise in the data and promote a clearer understanding of the patterns of response. However Study 6, which included both male and female participants, found that CBM procedures might not be suitable for all individuals regardless of gender. This implies there to be certain aspects of the relationship that might hold true for both genders. Naturally, further attempts to understand the relationship between biased cognitions and the stress response should devote time and effort into exploring the similarities and differences between genders.

As part of a self-report questionnaire measuring adherence to specific instructions relating to study involvement, participants were required to record any medication they were currently taking in all studies that included saliva sampling. One objective of this was to capture patterns of oral contraception use. Hormonal contraception has been shown to significantly influence levels of cortisol by changing binding practices, which results in more/less free cortisol (which is measureable in saliva) being present (Granger, Hibel, Fortunato, & Kapelewski, 2009). Further, use of hormonal contraception has recently been linked to a reduced cortisol response following a psychosocial stress task (Roche, King, Cohoon, & Lovallo, 2013).

Given the target population it seemed unfeasible to exclusively recruit non-contraceptive users. It was initially hoped that monitoring oral contraceptive use would afford understanding as to its statistical influence. However, the data collected for this purpose in the present studies is likely to be unreliable owing to extremely low reporting which, in a female undergraduate population, is highly unexpected (see Huber & Ersek, 2009). It is argued that, in this instance, the question was misphrased thereby leading to the majority of participants reporting only use of medication that is out of the ordinary in normal routines (e.g. antibiotics, psychopharmacological medications, or medications for chronic poor

health), rather than an exhaustive list of any medication. This appears to have been an unfortunate oversight and, as such, cannot be ruled out as an influencing factor in participant responses though it is hoped that the random allocation procedure that was adopted in all studies might at least have improved chances of equally weighted conditions.

9.5.8 Trend exploration. Throughout this thesis, trend interactions and main effects have often been explored where they relate to a-priori hypotheses. This has been completed in consideration of the accepted flexible license that accompanies a doctoral thesis compared to studies that are written exclusively for the purpose of publication. Where studies within this thesis have been independently prepared for peer review, a more formal approach has been adopted in line with standard scientific practice.

9.6 Conclusion

The findings of this thesis assist the field by helping shape our understanding of the processes by which cognitive biases operate and their limitations, the manners in which clinical tools might realign maladaptive tendencies, and by suggesting fruitful avenues for further research. Studies presented within this thesis did not document a robust link between cognitive biases and emotional vulnerability to stress, and attentional bias showed no relationship with physiological responses to stress. One possible explanation for not documenting such links is that the studies presented here successfully induced ‘real’ feelings of stress; overwhelming subjective capacity to cope with perceived demands. It is possible that, at this level, cognitive biases have less of an influence on when a stress response is initiated. This proposition is supported by the fact that, where previous studies have succeeded in documenting the bias/emotion vulnerability link using ‘stress’ tasks, they have induced only superficial representations of threat. As such, people who perceive threat everywhere (i.e. through having a negative bias) produce a stress response comparable to a

'real' stressor, while individuals with a more positive bias correctly deem the situation as benign. Further support for this notion arises from published studies that have effectively demonstrated the potential for one session of CBM to act as a cognitive buffer to the future perception of stress, which are also argued to have done so using a superficial stressor. In contrast, Study 5 failed to clearly show this link though was found to induce 'real' feelings of stress. Where 'real' stress has been successfully linked to trained biases previously, either using clinical samples or real-life stressors, studies have consistently implemented a more intense CBM training program involving multiple CBM sessions.

It is proposed that cognitive biases might influence initial vulnerability to stress by governing what situations are perceived as stressful. The stress response is primarily an adaptive process responsible for providing temporary relief to real threat. However, where biases become too negative, it is argued that individuals consistently perceive threat where none is present leading to excessive and unnecessary psychological and physiological activation. The resulting hyper-anxious state would thereby increase the risk to developing clinical disorders, while hyper-stimulation of the physiological stress systems would expose an individual to associated health-related risks, such as high blood pressure and future cardiovascular disease. Nevertheless, it should be noted that two independent studies presented in this thesis suggest that interpretive biases are involved in the recovery from such an episode of stress. This could imply that, following the establishment of a stress response, biases then operate on the magnitude of a response. The theory proposed in this thesis presents one possible account for the observed findings. In light of the extremely limited number of studies that investigate the influence of biases on the biological stress response, this theory would require significant further testing. Research might, for example, usefully be directed towards comparing predictive capacities of biases on the stress response to threatening and non-threatening situations to better understand the potential of this concept.

While the literature to date has considered CBM methods in terms of their ability to generally reduce emotional vulnerability, the research in this thesis can be argued to support a broader view of bias. Accordingly, under this notion, CBM might work by realigning maladaptive biases so that a stress response is triggered only for genuinely threatening events, rather than to benign events or to stopping it completely. If a lion appears in your path, failing to attend to it or failing to construe it as an imminent threat will probably result in negative consequences. Alternatively, failing to elicit appropriate escape responses due to an over-active (and thus exhausted) stress system will likely lead to an equally disastrous outcome. By rebalancing cognitions so that threats are only perceived when they present an actual danger, stress responses are acute and effective, and recovery following the episode is rapid and efficient, the physiological response can remain an adaptive process allowing you to do it all again the next time a lion crosses your path.

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APPENDIX I – In-House sAA Assay Development

Quantifying Alpha Amylase

The process of quantifying levels of alpha amylase was originally designed for purposes of diagnostics, with specific reference to pancreatitis. Early methods, such as the iodometric method (Wohlegemuth, 1908) and the amyloclastic method (Street & Close, 1956), which focus on measuring the disappearance of a substrate, have since been outdated with much simpler and more reliable methodology. A more recent method involves measuring alpha amylase through a chromogenic assay, which employs the use of the substrate 2-chloro-p-nitrophenol, which is linked with maltotriose. Together, these react with alpha-amylase resulting in a yellow coloured product that can be measured spectrophotometrically.

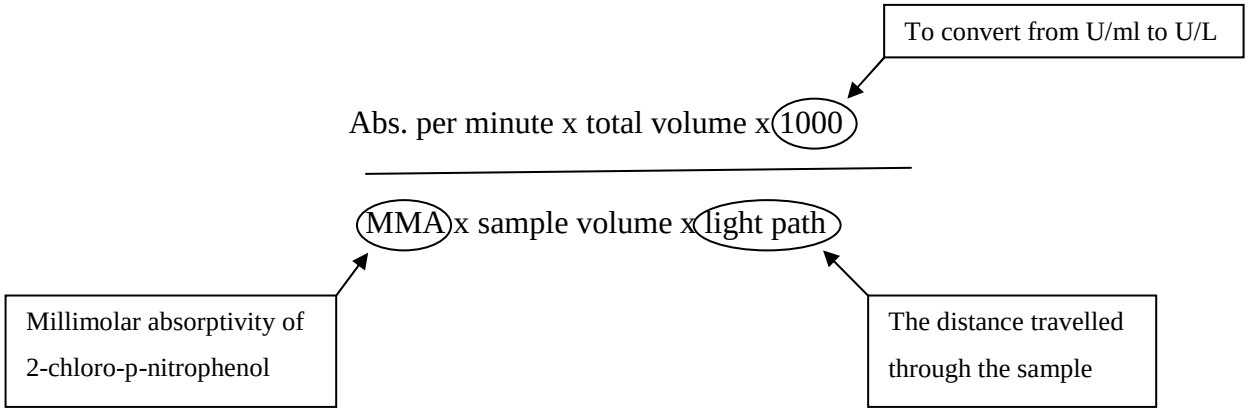
Similar to endpoint assays, such as an enzyme-linked immunosorbent assay (ELISA), the enzymatic reaction of amylase with the substrate is initially very fast before reaching a saturation point, which all samples achieve. However, unlike endpoint assays, due to the fact that the rate of change in optical density (colour) is directly proportional to amylase activity (Pointe Scientific, US), it is possible simply to infer concentration by measuring the early changes in the reaction. This prevents the need for standard measures to be assayed alongside unknown samples to correct any concentration inference as are necessary with ELISAs, which makes for ease of concentration calculation and a higher throughput by affording more available testing space on the microplate.

Many of the existing methods are based around using a cuvette spectrophotometer to measure the change in colour in the sample. Drawing on knowledge from existing commercial kits (e.g. Salimetrics LLC, USA), our aim was to establish an assay suitable for our research laboratory's microplate (MTP) system allowing many samples to be analysed

simultaneously. A second aim was to adapt the assay so that it could be automated by a Tecan Genesis Freedom (150/8) liquid handler.

Light Path

To measure the rate of the reaction between the substrate and alpha amylase, a spectrophotometer measures the intensity of the coloured by-product. For this, a spectrometer beams light through the sample well, a route referred to as the light path. As it passes through, some of the light becomes absorbed depending on the concentration of the analyte. The light is then diffracted into a spectrum, the optical density (OD) of which is measured at specific wavelengths by a photometer. Once absorbance per minute has been calculated using the delta OD (the difference between the first and second measurements), the following formula can be applied to obtain alpha amylase units per litre:



One key issue concerning our adaptation of this assay was the intention for it to be MTP-based and most MTP wells are relatively wide (thereby producing a shorter light path). As OD is proportional to light path, a longer light path would afford the assay sensitivity in detecting lower concentrations of alpha amylase. After reviewing the available options, a decision was made to use Greiner MTPs (clear, sterile, F-bottom polystyrene options; category number 655 161) as they seemed to best solve the problem above owing to their relatively narrow wells. However, this alone did not solve the problem entirely as it became

difficult to accurately measure the light path. This problem has not typically been encountered in previous use of the assay for purposes of clinical testing as diagnostic testing largely use cuvettes. As shown in Figure 34, cuvettes are typically singular with a fixed width of 1cm. When measured spectrophotometrically, this allows the light to travel horizontally through the cuvette with a known light path of 1cm. As MTPs have multiple wells arranged in columns and rows, the light path has to travel vertically through each well, and the light path is therefore related to the dimensions of the well and the amount of liquid present in each well.

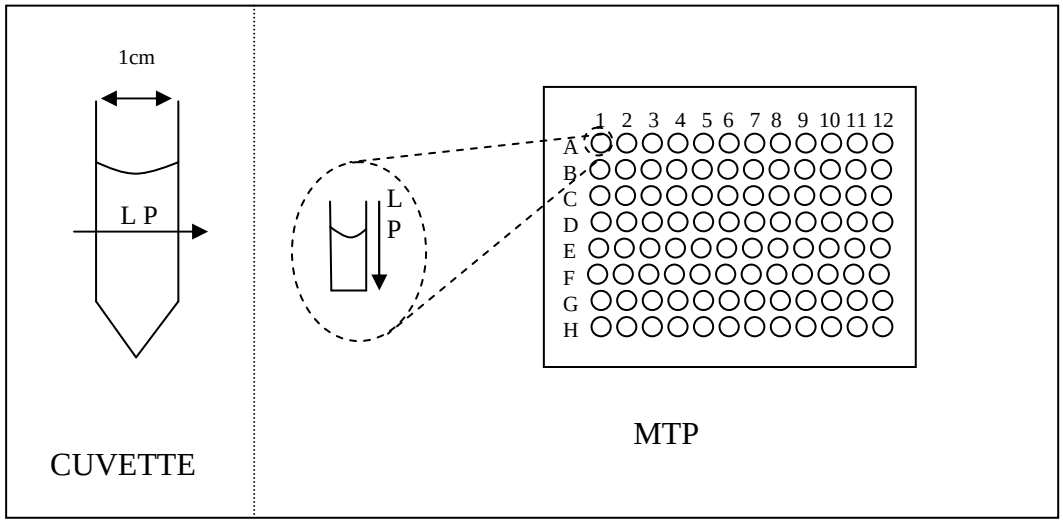


Figure 34. Comparison of a cuvette with an MTP. Note: LP = light path

The problem of measuring a vertical light path was further complicated by trying to account for any meniscus in the fluid. To overcome this challenge well dimensions, including the total possible volume, was ascertained from the MTP manufacturer prior to deconstructing an MTP and measuring the light path at the lowest point of the meniscus using 10x magnification for accuracy. The spectrophotometer was then set to read OD through the centre of the well to ensure the beam travelled through our measured light path.

Substrate Temperature Regulation

Conceptually, this assay is linked to fine temperature regulation. When the substrate is initially introduced to the sample, it must be heated to 37°C for optimal reaction. It is possible to source a water bath that is dedicated to heating to and maintaining specific temperatures in reagent troughs. While this provides an ideal solution, it is a costly one. Theoretically, an alternate solution would be to use an MTP-shaped bath to hold the reagent, which could then be heated in incubators designed to hold MTPs. Indeed, certain commercially available kits advise this (*cf.* Salimetrics LLC, USA). However their adaptation of this is too malleable and fragile for the mechanical assistance. Therefore, in a bid to simplify the procedure and also reduce wastage, a decision was made to customise a reagent plate (still based on the dimensions of a normal MTP plate) with four deep troughs capable of holding solution for three columns of the assay MTP, each with a v-bottom to minimise substrate waste (see Figure 35). This would enable us to use multi-shooting pipetting, whereby the liquid handler (LiHa) aspirates enough solution for 3 columns at a time to save time washing and re-aspirating the solution.

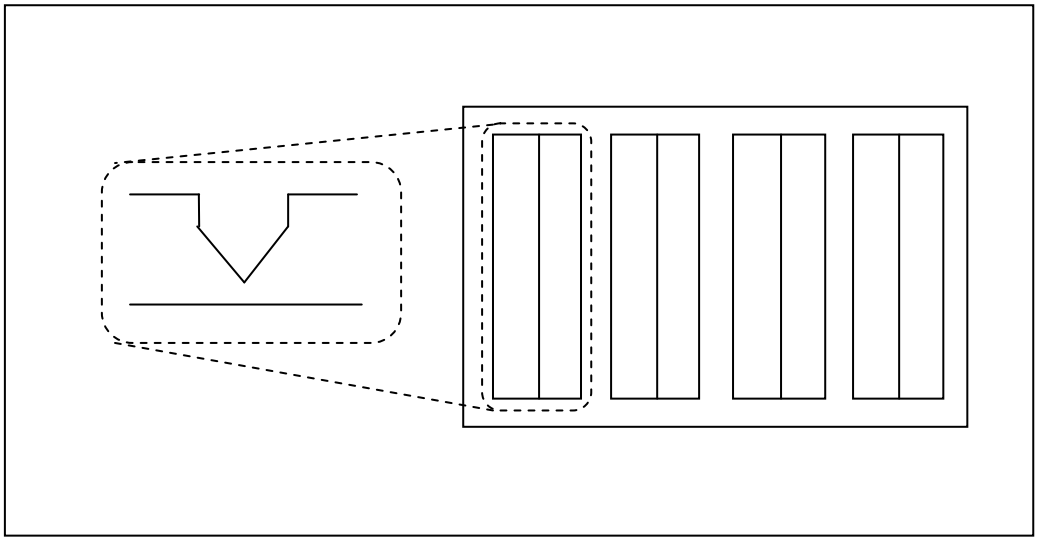


Figure 35. An illustration of the custom plate with four v-bottom troughs

As the LiHa has 8 tips that can work independently or simultaneously it is much faster than a human at pipetting. The robotic arm (RoMa) that moves plates around the deck follows set co-ordinated movements which makes it reliably replicable for each stage of the assay but can also make it much slower and less direct at moving plates around the deck compared to a human. Furthermore, it was necessary to amend the RoMa speed to avoid unnecessary disturbance of the substrate in the relatively exposed 4 troughs. As a result, the extra time for the heated reagent plate to be carefully transferred from the incubator to the deck caused a significant loss in temperature. The incubator was therefore set to overheat the substrate initially to 45°C (rather than 37°C) to account for this loss in temperature. As a further precaution, the assay MTP was also preheated prior to use to 39°C.

Pipetting

Small volumes. Whilst accuracy (i.e. actually pipetting the set volume) is obviously important, it is often considered more important to be precise. Precision involves being consistently reproducible and is measured as a coefficient of variation (CV) which is simply the averaged standard deviation of a number of events and is expressed as a percentage. The assay requires pipetting of 8µl diluted samples or known levels of alpha amylase (henceforth referred to as *controls*). Pipetting such small volumes demands both skill and focus and is notorious for producing unacceptably high CVs (above 15%). To aid pipetting, a human might use a tip touch technique, which involves holding the pipette tip to the side of the well after dispensing the solution to effectively wipe the tip clean. The LiHa is able to imitate humans in this way and observations demonstrate that all the tips do make contact with the appropriate wells. However early tests resulted in high CVs thereby suggesting the technique was not achieving adequate precision. Alternatively, a high velocity ejection method was opted for, whereby the samples are aspirated out of the tips at high speed followed by a quick stop which serves to break off any liquid clean from the tip.

Warm substrate. As previously discussed, with this kinetic assay it is crucial to capture the early stages of the reaction to determine the change in OD and, consequently, alpha amylase activity. As the LiHa undergoes a time-consuming rigorous tip-washing phase between each aspiration/dispense cycle to prevent sample contamination, it was calculated that the MTP would have to be read 12 times (once per column) in the spectrophotometer to ensure no sample achieved saturation before the second read. However, as the fixed tips have a capacity to hold 1ml liquid, and with the customised reagent plate holding enough solution for three columns, it should theoretically be possible to operate multi-shot pipetting whereby enough solution (3 x 320µl) could be aspirated and pipetted into three columns of the MTP at a time before having to return to the wash station. If successful, this routine would cut down our assay MTP reads three-fold from 12 to 4, which would substantially lessen overall running time as the reagent plate needed to be painstakingly returned to the incubator during spectrophotometer reads. However, during early trials it was noticed that during the process of aspiration for multi-shot pipetting, the reagent was cooling significantly. This was concluded to be due to the 960µl substrate being drawn into the tip lines, which were cold, meaning that (a) substrate was entering the sample well at less than 37°C, and (b) that the third column of each cycle had the most temperature loss resulting in observed drifts between the three columns. For this reason, a decision was made to revert back to single column pipetting and reads. As an additional measure, a tip-warming cycle was added where 320µl pre-heated substrate was aspirated and dispensed back into the trough once. This was practised once before each use of the reagent.

Final Assay Protocol

Method. Saliva samples are diluted 1:200 (to a readable range) in isotonic saline and mixed. Diluted samples and high and low controls (Clinical controls 1 and 2, category numbers C7590-50 and C7591-50 respectively, Pointe Scientific, US) are pipetted (8µl) into

the appropriate wells of a pre-incubated MTP (60 minutes at 39˚C). 320µl assay substrate (Liquid amylase reagent, CNPG3, category number A7564, Pointe Scientific, US) is added to one column from a pre-incubated MTP trough (60 minutes at 45˚C). The substrate plate is returned to the incubation pod before the assay MTP enters a plate reader (Tecan Infinite F200 microplate reader). The assay MTP is heated at 37˚C and shaken, and the relevant column is read spectrophotometrically at +1 minute and +3 minutes at 405nm (620nm reference read). The process is repeated until all 12 columns of the assay MTP have been covered.

Recent Additions

Although the process described above was entirely automated, it felt necessary to make part of the assay manual. Despite the lack of human involvement, the automated process took a considerable time to assay one plate owing to the manner with which the scripts were managed. The Tecan Genesis Freedom (150/8) liquid handler is controlled by two processes. Gemini is a piece of software containing the assay scripts and so is very precise, and is responsible for the dilution and pipetting stages. Alternatively, the flexible assay composer and task scheduler (FACTS) is a control centre responsible for scheduling the various processes and works to a more ambiguous timing agenda with occasional deliberate pauses, which is less suited to the final aspects of this assay where timing is crucial. The assay was therefore amended so that sample dilution and pipetting (controlled by Gemini) remained an automated process, but the introduction of the reagent and placement into the spectrophotometer was changed to become a manual (human) task. Reagent substrate was heated in an external incubator (still overheated). To pipette the reagent into all wells in an efficient manner, an electronic 1200µl capacity multichannel pipette (Biohit F1200, Category number 613-4113, Jencons) was used. The aspiration capacity of this pipette afforded one aspiration per trough of the reagent plate, to dispense over three columns of the

microplate each time. Tips were discarded after every third column (every trough of the reagent plate; four times per whole plate). Tips were still warmed through with one aspirate-dispense cycle as described before. This modification was found to be much faster, meaning that in the time that the robotic system had taken to measure one run (1 column), a researcher could measure an entire plate at once without the drift observed before owing to loss of heat in the substrate.

Precision Performance

Intra-assay precision. The intra-assay precision was determined by running the same samples down one column and then replicating that column with the same samples in the same row position across the remainder of the plate. High and low controls were used as test samples to additionally test that the plate remained within the assay range. Going down one column, high and low controls were placed in duplicate so that both the high and the low control had 4 representative samples down each column, each being replicated 12 times across the plate (see Figure 36).

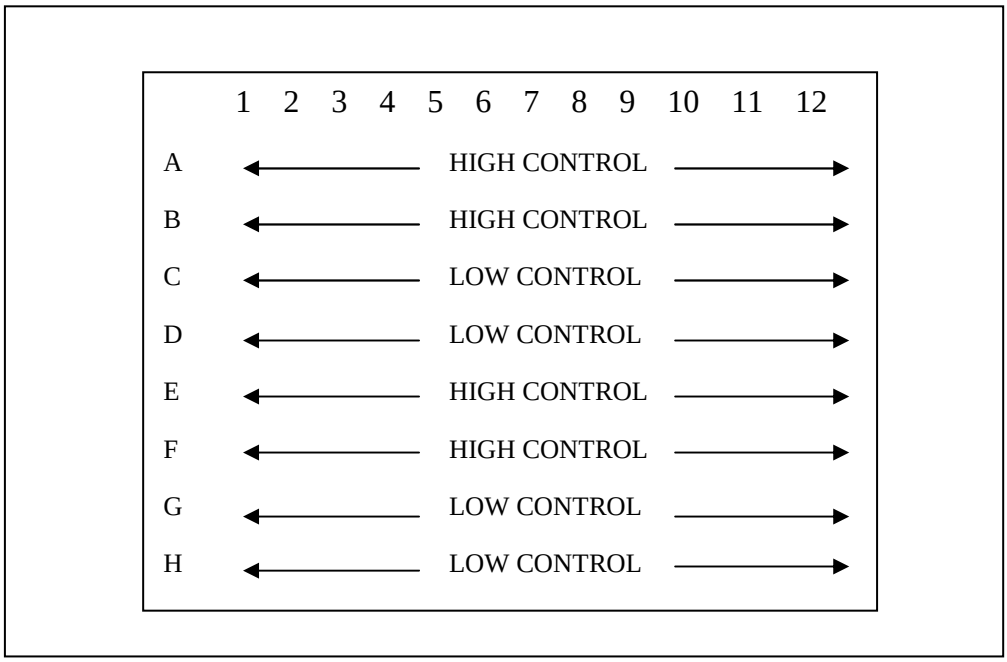


Figure 36. An illustration of the high and low control positioning across the intra-assay precision test plate

As can be seen from Table 24, the test produced good reliability, with all CV's below the generically accepted 15%.

Inter-assay precision. Data obtained from two runs using the same 80 samples but positioned in different and random locations each time was used to determine the inter-assay precision performance. The bottom row (H) was reserved for a constant control sample to check for drift across the plate, and high and low controls were assayed in duplicate in the final two rows to check the plate remained within assay range. Sample CV's averaged at 12.17 (*SD* = 10.84), again providing appropriate evidence of test-retest reliability of the assay. Statistically, the two plates were significantly related, $r(80) = .98$, $p<.001$, although the second plate did produce statistically higher results, $t(79) = -3.14$, $p = .002$.

Table 24.

Intra-assay precision performance data

		N	Mean (U/ml)	Standard deviation (U/ml)	Coefficient of variance (%)
HIGH CONTROL	*Across column A*	12	480.28	24.33	5.06
	Across column E	12	489.01	21.78	4.45
	Down row 1	4	455.90	13.05	2.86
	Down row 6	4	477.41	9.76	2.04
	Down row 12	4	498.13	15.03	3.02
	TOTAL	48	480.73	22.00	4.58
LOW CONTROL	*Across column C*	12	69.73	3.02	4.33
	Across column G	12	66.73	2.86	4.28
	Down row 1	4	64.12	2.55	3.98
	Down row 6	4	66.86	1.50	2.24
	Down row 12	4	71.16	2.02	2.84
	TOTAL	48	68.45	3.35	4.89

NB. Not all rows and columns are displayed in the table, just a representative sample. However 'total' refers to every possible high/low sample on the plate

Summary

To conclude, the existing assay protocol was successfully adapted to operate under specified conditions using a partially automated process. Several hurdles were overcome relating to automated pipetting accuracy at low volumes, the particularly sensitive nature of the assay, and efficiency to produce a reliable and useable assay with a high throughput potential.

APPENDIX II - Study 4: Attention Bias Test Stimuli

Table 25

Practice words for attention bias test

Neutral word	Emotional word
linear	heath
registry	estuary
depiction	causeway
tailoring	foothills
initial	island
soundtrack	evergreen
veteran	habitat
aspects	forest

Table 26

Buffer words for attention bias test

Neutral word	Emotional word
monopolies	breathless
pageants	skittish
chick	blush
economies	isolation
workplace	incapable
emission	disliked
proxy	loser
wider	worst
fax	shy
summaries	disgraced
inclined	dreadful
corners	awkward
gate	weak
ambassador	criticized
jute	wimp
linguistic	vulnerable

Table 27

Attention bias test 1 wordlist

Word Pair	Emotional word	Neutral word	Emotional word category
1	useless	lending	general
2	unwelcome	presenter	general
3	pathetic	loading	general
4	failure	clothes	general
5	ignored	raising	general
6	uptight	doorman	general
7	mistaken	theology	general
8	feeble	pumped	general
9	smothering	conductive	general
10	boring	employ	general
11	nervous	painted	sensation
12	vomiting	infinity	sensation
13	anguish	jackets	sensation
14	suffocating	repetitions	sensation
15	pain	mass	sensation
16	squeamish	lamplight	sensation
17	dizzy	foyer	sensation
18	lightheaded	exfoliating	sensation
19	shudder	coconut	sensation
20	tense	grows	sensation

Table 28

Attention bias test 2 wordlist

Word Pair	Emotional word	Neutral word	Emotional word category
1	inferior	advocate	general
2	mocked	orbits	general
3	despised	baseball	general
4	inadequate	electrical	general
5	abandoned	conducted	general
6	inept	towed	general
7	unstable	surveyor	general
8	ridiculous	quantities	general
9	stupid	occurs	general
10	apprehensive	multilateral	general
11	convulsion	excavators	sensation
12	embarrassed	transmission	sensation
13	intimidated	discounting	sensation
14	panic	canal	sensation
15	ashamed	gradual	sensation
16	tremble	rallied	sensation
17	sweating	allocate	sensation
18	hysterical	marginally	sensation
19	restless	rigorous	sensation
20	flustered	veritable	sensation

APPENDIX III - Study 4: Interpretive Bias Test Stimuli

Test 1 Scenarios, Comprehension Questions, and Recognition Statements

1) Category: Social interaction
   Title: The local pub
   Scenario: You are invited for a night out at a local pub, although you don't know any of the regulars very well. As you approach the door you can hear noisy conversation, but as you enter the room it becomes quiet.
   Comprehension question: Do you know most of the people at the pub?
   Negative foil: As you enter the room someone asks you why you are there
   Negative target: As you enter the room everyone stops and stares at you
   Positive foil: As you enter the room someone greets you warmly
   Positive target: As you enter the room the regulars stop their conversation and look over welcomingly
2) Category: Social interaction
   Title: Your wedding
   Scenario: You have invited some friends you have not seen for a while to your wedding and are awaiting their confirmation. You receive a card from one of them saying that unfortunately she will not be able to come, making you wonder about the turn out.
   Comprehension question: Are you only inviting close family to your wedding?
   Negative foil: Wondering about the turn out, you worry that the wedding invitations might get lost in the post
   Negative target: Wondering about the turn out, you think that not many of the old friends you invited will come to your wedding
   Positive foil: Wondering about the turn out, you get excited about starting to organise your table plan.
   Positive target: Wondering about the turn out, you think that many of the old friends you invited will come to your wedding
3) Category: Social interaction
   Title: Changing the return date on your coach ticket
   Scenario: You bought a coach ticket a while ago to visit a friend. You now would like to stay an extra day with them but are unsure about the company policies. You ring the

customer services number to change the return date. You can tell by the operator's tone of voice what they think about your request.

Comprehension question: Have you decided to change the date of your return coach ticket?

Negative foil: The operator says that the coach you have booked has been cancelled

Negative target: The operator seems annoyed by your request

Positive foil: The operator politely asks you whether you would like to take advantage of a special offer

Positive target: The operator seems friendly and sympathetic to your needs

4) Category: Social interaction

Title: The new sunglasses

Scenario: You have just bought some sunglasses that were on offer. You spent quite a long time to choose one pair as you generally do not wear glasses. When you arrive at a barbecue with your sunglasses on you notice quite a few people looking at you.

Comprehension question: Have you bought the most expensive pair of sunglasses?

Negative foil: Somebody comments that they saw those sunglasses cheaper in another shop

Negative target: People stare at you, thinking your sunglasses are not to their taste

Positive foil: Somebody comments that you have good taste and they would like to go shopping with you

Positive target: People look at your sunglasses, thinking how stylish they are

5) Category: Performance

Title: The first aid refresher

Scenario: You participate on a first aid refresher course at work. The instructor asks a question and no one in the group volunteers an answer, so he looks directly at you. You offer a reply, thinking about how your answer must be sounding to the others.

Comprehension question: Was the refresher course at your workplace?

Negative foil: You answer the question, realising you are irritated by this teaching style

Negative target: You answer the question, thinking how ignorant you may seem

Positive foil: You answer the question, pleased that you have such an interesting teacher

Positive target: You answer the question, thinking that the others may be quite impressed

6) Category: Performance

Title: Art club reunion

Scenario: You are at the yearly art club reunion and this is the first year you are presenting your work to people interested in art. When you finish your presentation you see some art critics near your painting and overhear what they are saying about your talk.
Comprehension question: Did you give a talk to some art critics?
Negative foil: You overhear some art critics saying that the art club lacks any skilled artists
Negative target: You overhear some art critics saying that your talk was somewhat lacking
Positive foil: You overhear some art critics saying they would like to buy one of your paintings
Positive target: You overhear some art critics complementing your talk

7) Category: Performance
Title: The group task
Scenario: You have been given a team group task as part of a selection process for an important job position. The task is difficult but you speak up with ways that perhaps could solve the task. When everyone turns to look at you, you can feel your pulse racing.
Comprehension question: Was the group task part of a company team-building event?
Negative foil: When everyone turns to look at you, you notice that they are all much better dressed than you are
Negative target: When everyone turns to look at you, you feel nervous about explaining your idea
Positive foil: When everyone turns to look at you, the interviewer comments he is delighted that somebody has had an idea
Positive target: When everyone turns to look at you, you are excited about explaining your idea

8) Category: Performance
Title: Your puppy
Scenario: You are walking your puppy on the lead but it is showing too much excitement when seeing other dogs in the street. It has just tried to jump at someone walking their dog and they turn to say something to you.
Comprehension question: Are you walking your puppy on a lead?
Negative foil: The dog-walker says she was recently bitten by her dog
Negative target: The dog-walker turns angrily to say that you should have more control over your puppy

Positive foil: The dog-walker approaches your puppy from the other side of the path and says she loves that breed

Positive target: The dog-walker stops to say how nice it is to see such a friendly puppy

9) Category: Performance

Title: Writing an important report

Scenario: You are at work writing an important report when a colleague with a senior position comes into the office. You can see they are behind your back looking at your work and you are wondering what they are thinking.

Comprehension question: Did a colleague come in to your office?

Negative foil: Your colleague sees over your shoulder that you are checking your personal email instead of writing the report

Negative target: You imagine your colleague thinks you have not written very much of the report so far

Positive foil: Your colleague brings you a cup of coffee and tells you that you deserve a break from writing the report

Positive target: You imagine that your colleague is impressed by how much of the report you have written so far

10) Category: Performance

Title: Bowling with colleagues

Scenario: You are bowling with your new colleagues from work. Your team is slightly behind and all eyes are on you when you take your turn. You throw the ball and feel it slide from your fingers. As you walk back to your seat you can see your team's facial expressions change.

Comprehension question: Was your team slightly ahead when you stood up to take your turn?

Negative foil: When you walk back to your seat, you overhear your colleagues complaining that they are bored

Negative target: When you walk back to your seat, your colleagues look disappointed by your performance

Positive foil: When you walk back to your seat, your colleagues tell you that your team has won

Positive target: When you walk back to your seat, your colleagues look impressed by your throw

Test 2 Scenarios, Comprehension Questions, and Recognition Statements

1) Category: Social interaction
   Title: The house-warming party
   Scenario: Your new neighbour invites you to their house-warming party. You arrive to find many other guests whom you do not know. You try talking to some of them and by their reactions you see how they find your conversation.
   Comprehension question: Was the party at a relative's house?
   Negative foil: You don't know anyone there and everyone ignores you completely
   Negative target: You talk to some guests but they think what you say is boring
   Positive foil: You meet many guests whom you know and enjoy talking to them
   Positive target: You talk to some guests and can tell that they find you interesting
2) Category: Social interaction
   Title: Shopping in the city
   Scenario: You are going shopping for the weekend in the city where an old friend you haven't seen for years lives. You'd like to suggest to meet up but are unsure as it is very short notice. You give them a call and their phone rings for a while. When they eventually pick up the phone and you start to talk you feel butterflies in your stomach.
   Comprehension question: Are you going to be doing some shopping over the weekend?
   Negative foil: As you ask your friend about meeting up, they rudely interrupt
   Negative target: As you ask your friend about meeting up, you are nervous because you expect they will say no
   Positive foil: Your friend suggests you have a picnic together
   Positive target: You are excited about asking to meet your friend and think they would like to see you
3) Category: Social interaction
   Title: Dinner in a restaurant
   Scenario: You have ordered an elaborate dish with a creamy French sauce. When they serve you the dish the sauce looks to you all curdled. You hesitate whether to call the waiter as they look busy but in the end you tell them and their reaction is unexpected.
   Comprehension question: Was your dinner served with an Italian tomato sauce?
   Negative foil: When you called him over, the waiter was arguing with another customer
   Negative target: The waiter is irritated by your comments and tells you the sauce is meant to be that way

Positive foil: All of the waiting staff in the restaurant are well-dressed and friendly

Positive target: The waiter is very apologetic about the sauce and goes to the kitchen to find you an alternative dish

4) Category: Performance

Title: Photos of your flat

Scenario: You have placed some pictures of your refurbished flat that is on sale on the web after someone who seemed interested requested a look. You are now looking through messages and are taken aback by the thread some people have posted.

Comprehension question: Have you placed some photos on the web of your car?

Negative foil: You are surprised that nobody has commented on the pictures on the website

Negative target: You are surprised to find many negative comments about your flat

Positive foil: You are surprised that a good friend has posted a message on your website

Positive target: You are surprised by all the positive comments about your flat

5) Category: Performance

Title: School staff meeting

Scenario: You have just started organising after-school activities as part of your teacher training. In a school staff meeting with your senior colleagues it is now your turn to propose an activity. You quickly say the first idea that comes to mind which prompts a lot of remarks.

Comprehension question: Were there senior colleagues at the staff meeting?

Negative foil: Your colleagues remark that they are concerned about your teaching style

Negative target: Your colleagues remark that your proposed activity is inappropriate

Positive foil: Your colleagues remark that you have been doing very well in your training so far

Positive target: Your colleagues remark that your proposed activity will be fun and popular with the children

6) Category: Performance

Title: The wedding reception

Scenario: Your friend asks you to give a speech at her wedding reception. You prepare some remarks and when the time comes get to your feet. As you speak, you notice some people in the audience start to laugh.

Comprehension question: Did you stand up to speak?

Negative foil: As you speak, some people in the audience start to yawn in boredom

Negative target: As you speak, people in the audience find your efforts laughable

Positive foil: As you speak, people in the audience applaud your comments

Positive target: As you speak, people in the audience laugh appreciatively

7) Category: Performance

Title: Scout camp

Scenario: You are an adult helper at a large scout camp. On the first week you are placed with a group of people you barely know to organise the talent show. In the spur of the moment you decide to give some input. Everyone's eyes turn to look at you while someone in the group tells you their opinion.

Comprehension question: Did you choose who was in your group?

Negative foil: When everyone turns to look at you, you notice that some of the other helpers look unwell

Negative target: Someone in the group criticises your idea for the talent show

Positive foil: When everyone turns to look at you, you notice how friendly and energetic they seem

Positive target: Someone in the group tells you they like your idea for the talent show

8) Category: Performance

Title: In the supermarket

Scenario: You are in the supermarket doing the shopping with your young child. You are at the cashier point queuing up when they start jumping around and laughing. You hear the cashier make a comment about your child to their colleague as you pack the bags which makes you think about your parenting.

Comprehension question: Did you bring your child shopping with you?

Negative foil: Your child knocks over a basket of shopping and the cashier looks angry

Negative target: The cashier comments to their colleague that your child is badly behaved and that you should control your child better

Positive foil: Your child helps you with the packing and tells you they like shopping with you

Positive target: The cashier comments to their colleague that your child is very happy and that you are obviously a good parent

9) Category: Performance

Title: A report for your new manager

Scenario: You are working with a manager that you have never worked with before. You are writing a report for them and have been putting in a lot of effort. Your manager emails

you some thoughts on your report and when you read it you are surprised that they have made so many comments.

Comprehension question: Did your manager telephone you to tell you their thoughts?

Negative foil: Your new manager is not pleased with how you are organising your time

Negative target: You see some comments from your new manager suggesting that your work leaves a lot of room for improvement

Positive foil: Your new manager is very satisfied with your motivation and efficiency

Positive target: You see from the comments that your new manager is acknowledging the excellent work you have been doing

10) Category: Performance

Title: The online course

Scenario: You enrolled on an online course to get a professional qualification. You have been studying for the final test which is in two days time in quite a relaxed way. To have a sense of how your revision is going you and other coursemates try an online sample test and you realise how your revision strategy was.

Comprehension question: Is the final test in two days time?

Negative foil: You cannot access the online sample test because there is a problem with your computer

Negative target: The results of the online sample test show that your revision strategy was unsatisfactory

Positive foil: You are very pleased with the online course and decide to recommend it to a friend

Positive target: You do well in the online sample test showing that your revision strategy was very good

APPENDIX IV – Study 4: OCam scripts

Directions are in brackets. Researcher refers to the part of the actual researcher (KR/CP), and colleague refers to the confederate researcher on the videos. Participant 1 and 2 (videoed) already say in front of the camera.

Neutral Video

Colleague:	Hi
Experimenter:	Hi, can you hear me OK?
Colleague:	No, not really, I'll turn up the volume.
Experimenter:	OK (*pause*) Is that better?
Colleague:	Yes. (*Directed at Participants 1 & 2 only*) Ok, I have already explained the details of what you will be doing today, but the experimenter will quickly run through it again with you now. (*Look at camera*) I'll leave you to it. (*Walks off camera*)
Experimenter:	OK, thanks. (*To Participants 1 &* 2) Could you move in a bit, please, you're a bit off camera?
Participant 1 & 2:	(*Both move chairs closer to the monitor and both look at the camera*)
Experimenter:	As you all know we are not using your names for confidentiality reasons, so you have all been assigned a number. So you are participant 1, you are participant 2 and you are participant 3 (*Gesturing to the participants*)
Participant 1 & 2:	(*Both smile and wave*) Hi
Experimenter:	(*Run through the instructions for the study*) To ensure everybody is given the same instructions during the study, I am going to read the experimental statement to you once more. As you all know this experiment aims to analyse how communicating through web-chats affects the development of new social ties and impression formation during a brief and very structured interaction. You have all prepared some points to share. Participant 1 will go first, then participant 2, then participant 3. (*Gestures to participants in turn)* You all need to consider your thoughts and feelings during the conference, as

you'll each be asked questions on this later. You will each have a turn to talk. You are not to ask questions, just listen to what each has to say. If you wish to comment on what someone else has said, you may do so during your turn to talk. When you have finished say "that's all", then the next person can start their turn. I know that it could be a bit boring taking part in two web-chats in a row, but please try not to get distracted and do concentrate on the web-chat while the others are talking. Ok, I'll leave you for about 5 minutes to give you some privacy. Participant 1, you can start when I leave. (*Leaves the room*)

(Total time to give instructions is 1 minute 5 seconds)

Participant 1: (*Scratches face 5 seconds before they are about to begin talking = sign for researcher that they are about to start your talk*) Ok... (*begin talking for approximately 1.5 minutes, select a topic*) ...That is all.

Participant 2: (*Talks for about 1.5 min select a topic. At the end says the following*) Well that's all I can think of.

Experimental Subjects turn to talk.

Participant 1 & 2: (*Both to smile and nod sometimes, as if engaged by listening to someone talk. Look directly into the camera with occasional glances away etc.*)

Experimenter (*After 1.5 minutes of the experimental subject talking*) Ok, that's fine. Thank you very much. I am going to cancel the conference connection now. The three of you can start the questionnaires, which should come up on the screen. (*Appears to cancel connection.)*

Ostracism video

Colleague: Hi

Experimenter: Hi, how's everything going?

Colleague: Yes, everything's going really well at the moment. *(Walks off camera*)

Experimenter: Oh good... (*interrupted by Participant 2 coughing*) ...Oh dear, are you OK?

Participant 2: (*Nods*) Yes, I'm fine thanks.

Experimenter: Ok, so let's start the next web-chat. We keep the same numbers for this conference. So you are participant 1, you are participant two and you are participant three (*Gestures to the participants*)

Participant 1 (*Both smile and wave*) Hi

& 2:

Experimenter: OK, great, I'll leave you to it. Participant 1 you can start when I leave (*Leaves the room*)

Participant 1: (*Wait 5 seconds, then talks for approximately 1.5 minutes on selected topic*) That is all.

Participant 2: (*Talks for approximately 1.5 minutes on selected topic*) Well that's all I can think of.

Experimental Subjects turn to talk.

Participant 1 & 2: (*Look directly into the camera smile and nod, as if engaged by listening to Participant 3 with occasional glances away etc.*
After 25 seconds participant 2 starts to look away, looks slightly bored.
After 30 seconds (from start) participant 1 starts to look away, looks bored.
After 35 seconds participant 2 asks a question to participant 1 in a low voice, participant 1 nods shortly to participant 2 and focuses again on the real participant.
After 45 seconds participant 1 starts a separate conversation with participant 2 by asking a question which refers to what participant 1 said)
(*Continue conversation until colleague interrupts*)

Experimenter (*After 1.5 minutes of the experimental subject talking*) Ok, that's great, I'll just stop you there and then you can complete your next questionnaires (*appears to cancel connection*)

Inclusion Video

Experimenter: Hi, are you ready for us?

Colleague: Yep, we're ready to start, over to you (*Walks off camera.*)

Experimenter: OK, great, so we'll keep the same numbers for this conference. So you are participant one, you are participant two and you are participant three *(gestures to the participants)*

Participant 1 & 2: (*Both smile and wave*) Hi

Experimenter: OK, I'll leave you to it. Participant 1 you can start when I leave (*Leaves the room*)

Participant 1: (*Wait 5 seconds, then talks for approximately 1.5 minutes on selected topic*)

That is all.

Participant 2: (*Talks for approximately 1.5 minutes on selected topic*) Well that's all I can think of.

Experimental Subjects turn to talk.

Participant 1 & 2: (*Both smile and nod more than in the neutral condition, as if engaged by listening to Participant 3 talk. Look directly into the camera with occasional glances away etc. Both are more friendly and attentive than in the neutral condition*)

Colleague: (*After 1.5 minutes of the experimental subject talking*) Ok, time is up, this was the last web-chat. Thanks for participating. (*Asking informally*) What do you think of this conference-system?

Participant 1: It was interesting to do, and good to talk to Participant 3 (*Gestures to camera*) who seems really down to Earth and I think we'd get on.

Participant 2: (*First looks at Participant 1, nodding, then to Participant 3, smiling and nodding*) Yes, it was quite nice. I also think we'd get on well.

Colleague: OK, good, well I'll cancel the connection and then you can all start your next questionnaires. (*Video ends.*)

Experimenter: (*After a little time has passed, re-enters the room.*)

APPENDIX V – Study 5: CBM-I Training Stimuli

All participants were presented with the same scenarios. Positive CBM-I always drew on the positive resolution of the scenario, while sham CBM-I drew an equal amount of times on the positive and negative interpretations. Positive disambiguations of the scenarios below are presented first, followed by the negative alternative.

CBM-I Scenarios

1) Scenario: You are taking a mock driving test and your instructor takes you on a difficult route. You think that you might have made a number of mistakes and at the end your instructor tells you that had it been for real you would have failed. When you ask others who had the same instructor you find out they were all told they would have f - - l – d (failed) / s u - - e - d - d (succeeded)
   Comprehension question: Did you do just as well as the other students?
2) Scenario: You are taking a test and find it very difficult. When the results come out you find out that you scored under 50%. You ask your fellow students how they did and find out that everyone else's mark was l - w - - (lower) / h - - h - r (higher)
   Comprehension question: Was your mark higher than the other students?
3) Scenario: You overhear a radio programme about performance on a test you completed recently, but not very successfully. An eminent academic explains that new test techniques are a better way of testing students' ability and a student of average ability should expect to get most of the questions w r - - g (wrong) / c - r r - - t (correct)
   Comprehension question: Did you do as well as you would have expected to do?
4) Scenario: You are playing party games with your friend's children as it is their birthday. They play a game where they have to remember lots of things in their head at once. You take part but quickly realise that you are finding the game quite hard. You stop playing and help to tidy up. For you, not being able to remember the items is
   i n c o - s - q - - n t - a l (inconsequential) / w o - - y - n g (worrying)
   Comprehension question: Does it bother you that you cannot remember the items?
5) Scenario: You are taking part in paintballing as part of a team building exercise at work. Everytime you start a new game you get shot straight away. Thinking that you could well be the worst on your team makes you feel like l - - g h - - g (laughing) / c r - - n g (crying)
   Comprehension question: Does being the worse on your team upset you?

6) Scenario: Some friends of yours encourage you to join them in taking the exam which determines your ability to join the civil service. The questions are all very abstract and challenging and you find out that you had a low score. They know how this will make you feel given that your own career interests lie e l - e w - e r e (elsewhere) / t - e r e (there)
   Comprehension question: Does it matter that you didn't do well on the test?
7) Scenario: You are comparing notes about your children with your brother and he says that his children are doing rather better at sport than yours. You realise that one reason is that your children do not attend extra evening coaching which means that they are less p r - s s - r – d (pressured) / a d - - n c - d (advanced)
   Comprehension question: Do you think that more coaching would improve things for your children?
8) Scenario: You are attending a viewing of new abstract paintings, and overhear the person who runs the gallery explain why they are important. You feel you don't understand the paintings and decide that the person talking is trying to show how clever they are. You think that compared to them you are quite k n - - l e d g - - b – e (knowledgeable) / i n f - r i - r (inferior)
   Comprehension question: Do you know quite a lot about art?
9) Scenario: You have a series of reasoning tasks to do and are then given a challenging one. You concentrate hard but it is still very difficult and when you finish you know you didn't get many correct. Later it is explained that the test assessed your r e - o - v e (resolve) / i n t - l l - g - - c - (intelligence)
   Comprehension question: Was your score an important part of the test?
10) Scenario: At your evening class, you are given a task to complete for the next week. You finish it early and ask the tutor for his opinion. He says the work is good, apart from missing a section. You feel that he will think you are l e a - - i – g (learning) / c - r e l - - s (careless)
   Comprehension question: Was your tutor pleased with the quality of work on your paper?
11) Scenario: As you struggle to solve even a few of the items in a test, you recall a previous occasion when you also found the tasks nearly impossible. On that occasion, afterwards you had compared notes and found that compared with you, others were considerably more c h a - - e - g – d (challenged) / c l - v - r (clever)
   Comprehension question: Did you do better than the others on the previous occasion?

12) Scenario: A friend suggests that the two of you join an evening class on creative writing. At the early stages the teacher finds lots of points to suggest improvement in your work and you are asked to discuss these points in class. You are all beginners together but you think the others will find your mistakes r e - s s u r - - g (reassuring) / g l a - - n g (glaring)
Comprehension question: Did you think other people would be superior?

13) Scenario: You are attending a session where you are being taught the difficulties of creating a good test of cognitive ability. The lecturer explains how it is necessary to try out the test on a range of people to get the correct level of difficulty. To make sure that a wide range of abilities can be assessed it might be necessary to include a number of tasks that most people find quite p - - b l - m - t – c (problematic) / s - - p l e (simple)
Comprehension question: Are some of the tasks intended to be quite easy?

14) Scenario: You receive an essay back from your tutor and you got a much lower grade than you expected. She tells you that on this occasion she deliberately set a task that you would find h a - - (hard) / u n d e - - n d - - g (undemanding)
Comprehension question: Did your tutor expect you to find it difficult?

15) Scenario: You are goal keeper for your local pub football team. You save some of the goals but let a number in. At the end of the game the score is 6-5 to y - - (you) / t - e m (them)
Comprehension question: Did you win the game despite your mistakes?

16) Scenario: You are at a party at the house of a neighbour who is very keen on general knowledge games. He insists that you take part in a new game that he likes. You can answer only a few of the questions and feel a bit embarrassed. Then you think back to the ones you answered and feel quite p r o - - (proud) / i - n o r - n t (ignorant)
Comprehension question: Did you end up feeling pleased about being able to answer a few the questions?

17) Scenario: You are visiting your bank to check on your account. You have added up all the transactions in your recent statement and think that there has been an error. When the clerk goes over the items with you it is clear that the mistake is t - e i r – (theirs) / y - - r s (yours)
Comprehension question: Did you make a mistake adding up the transactions?

18) Scenario: You take night school classes in order to get a GCSE in German. Before the final exam you went to Germany for a couple of weeks with the idea of brushing up on your skills. When you get the exam results you find out that you just passed even though you spent all of your time in Germany r e - - x i n – (relaxing) / r e - - s i n - (revising)

Comprehension question: Did you expect to do better in the exams?

19) Scenario: You apply for a job and are asked to take part in some tests as part of the recruitment process. It is very important and you try your hardest but you find the different tasks very difficult. At the end their feedback makes you think that this exercise revealed your w - l l p - w – r (willpower) / f - a w - (flaws)
Comprehension question: Was the exercise making an assessment of your intellectual ability?

20) Scenario: You are playing a solo as part of a concert. As you are playing you know you are makingsome mistakes. At the end you think back to the bits that you played well and feel p l - - s – d (pleased) / a s h - m - d (ashamed)
Comprehension question: Do you feel happy when you think about the bits you played well?

21) Scenario: Together with some colleagues you are given a new job to do at work. You think you have some idea how to do it, but when you try it on your own nothing works out right. Feeling stupid you ask your colleagues and discover that compared to you they are c l - - l - s s (clueless) / k n o - l - - g e a b - e (knowledgeable)
Comprehension question: Were the others just as confused as you?

22) Scenario: You know that you have answered very few questions correctly in the test you have just taken. As you think about other people doing the same test you guess that they will think that compared to them you are rather b e - t - - (better) / a - f - l (awful)
Comprehension question: Will the others doing the test approve of your results?

23) Scenario: At a party you overhear two teachers talking about class exercises they have recently set. One explains that in the first session he gives new groups an almost impossible test, and reads their scores aloud to allow them to compare the results. He then leads a discussion and uses this feedback to encourage them to treat the results as a j o – e (joke) / j o - t (jolt)
Comprehension question: Are the results of the test important?

24) Scenario: You meet some old friends and begin discussing your current ambitions. At college, you had all met with same the career advisor who set you a series of tests to assess your aptitude for different jobs. Thinking back to how well you did on these tests, and your subsequent career choice, makes you realise that your scores on each one were clearly i r r - l - v - n t (irrelevant) / s i g - - - i c a - t (significant)
Comprehension question: Were your scores on the tests important?

25) Scenario: You are socialising with your partner's family and their nephew makes everyone join in with a game he is keen on. You are generally not at all efficient and get an extremely low score. From the expression on the others' faces you see that getting a higher score for this game might make them think you were rather a g - - k (geek) / s t - - (star)

Comprehension question: Did you want to get a high score on the game?

26) Scenario: A group of colleagues invite you to a social evening with a quiz as part of the entertainment. You are part of a team but cannot answer all but one or two questions. Afterwards when they discuss how successful it was they comment on the specialist questions that were asked. You can see that your correct answers were v - t – l (vital) / i n s - f f - c - e - t (insufficient)

Comprehension question: Were your friends pleased with your performance?

27) Scenario: You are playing pool after work. You are offered a game with someone you have not met before and thinking that you are rather good you start to play. You manage to pot a couple of balls before you lose and then find out that the person you were playing against was a p r - f - s s - - n – l (professional) / b - g - n - e r (beginner)

Comprehension question: Would you have expected to win the game?

28) Scenario: You are reading a self help book about how to impress at work. One of the chapters is about coping with setback. You try to implement the suggested strategy as you read that you should now imagine making errors in an important task. As you think about this situation you find your mind is filled with a feeling of c a - - (calm) / i n a - e q u - - y (inadequacy)

Comprehension question: Do you feel relaxed?

29) Scenario: A colleague asks you to organise a rota for coffee making duties. As you pin it up, several people complain about the way in which you have designed it. You reflect on their comments and conclude that your organisational skills are probably f i - - (fine) / s l - - p y (sloppy)

Comprehension question: Are you satisfied with your organisational skills?

30) Scenario: Your orchestra asks you to play a solo at the next concert. You practice a few times until you feel ready to play it with the orchestra. At the first rehearsal you make a mistake. The conductor will think that your work is p r o - i s - - g (promising) / r - s h - d (rushed)

Comprehension question: Did you feel disappointed with your performance?

31) Scenario: You have signed up for a study in which you are asked to complete some tests of ability. As you read the instructions and begin the first task you realise that it is difficult, although it is clearly designed so that someone of average ability should find most of the examples t - i c k – (tricky) / s t r - - g h t f - - w - - d (straightforward)
Comprehension question: Is the task designed so that the examples are easy?

32) Scenario: You are trying out a memory puzzle in a magazine you are reading. At the end you did not get many answers correct. You read to the end of the article, and it explains that the difficulty has been set so that on most items someone of your general ability would be m - s t - k – n (mistaken) / r - - h t (right)
Comprehension question: Did you make more mistakes than would be expected?

33) Scenario: You are being assessed for promotion at work and have a tough interview to go through. After each answer you are told what you should have said and get the impression that you are failing. At the end you are told the questions were deliberately challenging and that your promotion has been a - - e p t - d (accepted) / d e - - i n e d (declined)
Comprehension question: Was your impression about failing wrong?

34) Scenario: You are at a party and one of the games being organised is a trivia quiz. You feel obliged to join in, but do not know the answers to any of the questions you were asked. You think that the questions were mostly about things that are interesting to people who are relatively u n - n t e l l - g - - t (unintelligent) / s - - r t (smart)
Comprehension question: Did you care about not knowing the quiz answers?

35) Scenario: You are curious about joining MENSA, the society for people who score highly on intelligence tests. You take their test and are told that your score was not high enough to be accepted. Thinking about it you realise that people who eventually join must be very c o n c - - t – d (conceited) / i n s - - r a t i - n a l (inspirational)
Comprehension question: Are you unhappy about not being accepted?

36) Scenario: You join a tennis club and before long, you are asked to play in a doubles match, even though you are very nervous. You lose and afterwards you discuss your performance with your partner. They focus on the shots that you played w – l (well) / s h - d d - l y (shoddily)
Comprehension question: Did they focus on your good performance?

37) Scenario: You have gone on a skiing holiday with friends. You take part in a downhill race with the other 10 students in your class. You finished sixth even though you had spent more time than anyone else p a r - y - n g (partying) / p r - c t i - i - g (practicing)
Comprehension question: Were you relatively pleased with your position?

38) Scenario: You are devising a short course for teachers. You plan to set a really difficult test early in the course to encourage them think about the emotional responses to such tests in their future students. When you explain how the test was designed as a teaching tool you emphasise that its actual capacity to estimate ability is really n - g l - g - b l e (negligible) / a c c - r - t - (accurate)
Comprehension question: Is it a good test of ability?

39) Scenario: Reflecting one day, you look back at achievements and disappointments that you have experienced during your life. Overall, your main feeling about life so far is one of s - t i - f a c - - - n (satisfaction) / r - g r e - (regret)
Comprehension question: Are you generally happy about the events experienced in your life?

40) Scenario: Your bathroom looks rather dingy and so you decide to put new tiles up. You are not experienced and when you look closely they are not all straight. In the end, you look at your work and decide that your efforts were w - - t h - h i – e (worthwhile) / f - t - l e (futile)
Comprehension question: Did your efforts improve the look of the room?

41) Scenario: As you work at each new example in a test you find you are not able to solve them in the time given. You assume that you should be able to do the tasks and the time allowed has therefore been carefully chosen so as to be i - p - s s - b - - (impossible) / e - o u - h (enough)
Comprehension question: Do you think you should finish in the time?

42) Scenario: You are given a modern test of intelligence, with separate sections to assess different abilities. You work through trying your hardest but find the tasks very difficult. At the end of the session the assessor suggests that someone from your background should expect to get only about 10% of these items a - - u r a t – (accurate) / w - - n g (wrong)
Comprehension question: Were you expected to get most of the items wrong?

43) Scenario: You are set to work on a test that has two components. Each element is fairly easy by itself but you have to do both together. It is surprisingly difficult to solve anything when you have so little time to think and you make many mistakes. You think that this number of mistakes is not s - - p r - s - n g (surprising) / n - - m - l (normal)
Comprehension question: Do you think you were worse than others at this test?

44) Scenario: You have a go at an online game that your friend says is really fun. You start playing but don't seem to get a very high score. You think that this is because you find the game too t r - v - - l (trivial) / c - - p l i - a t - d (complicated)
Comprehension question: Was the game too difficult for you?

45) Scenario: On holiday, one evening your family group is persuaded to take part in a team quiz somewhat against your better judgement. With your low score you earn the loser's prize; a mug for each team member. Compared with the ornate winner's trophy you think that being given mugs will be really u - e f – l (useful) / e m - a - r a - s - n g (embarrassing)
Comprehension question: Are you glad that you were given the mug?

46) Scenario: Generally when you take part in cognitive ability tests you feel you do reasonably well. This time, you can hardly solve any items. You hear that you are being directly compared with another group of people who have done much better. Their success is down to their having much more specific and extensive t - - t i o n (tuition) / a b - l - t - (ability)
Comprehension question: Are the other group more intelligent than you?

47) Scenario: You are feeling rather tired and decide to have a go at a crossword in the paper. You try for a while but cannot make sense of any of the clues. In the end you give up thinking that in order to do well at something like this you would need to be more a - e r t (alert) / i n - e - l i - - n t (intelligent)
Comprehension question: Did you think you weren't clever enough to solve the clues?

48) Scenario: You take part in a research task and are surprised at your score at the end. It is explained that the task was designed to so that this score would reflect your ability on the subset of the scale measuring an aspect of 'performance'. Your responses at the end of this part of the test assesses whether you have good ability to deal with task f - - l u r – (failure) / s w - t c h - - g (switching)
Comprehension question: Was this testing your ability to deal with disappointment?

49) Scenario: After trying the first few questions in a test task you realise that most of these items stretch your ability. As you tackle the ones that follow you feel d - t - r m - n – d (determined) / d e m o - - l - s - d (demoralised)
Comprehension question: Are you looking forward to tackling the next items?

50) Scenario: The morning of your first appraisal with your new boss has arrived. She has a reputation for going over fine details of other people's work. She points out some of your

mistakes and weaknesses and you think she will follow this up with other comments that are more c - - p l e - - - t a r y (complementary) / c r i - i c - l (critical)

Comprehension question: Does your new boss have anything nice to say about your work?

51) Scenario: As you work through the examples in a test you have been set, you find it hard to keep all the details in mind to answer the questions. Then you think about your performance in this type of test and you realise your achievements compared with other people will be very r - - s o n - b l e (reasonable) / f - - b l - (feeble)

Comprehension question: Do you think you are performing worse than most others on this test?

52) Scenario: You have taken an exam as part of an evening course and don't feel you did well. At the next class the grades are on the notice-board and everyone is looking at them. As you compare your grades you realise everyone else did w - - s – (worse) / b e - - e - (better)

Comprehension question: Did you do better than everyone else?

53) Scenario: As part of an intelligence test you have to solve word encryption codes. Although you expected to do more, at the end of the time you have solved only one. You conclude that a higher score is more u n - s - a l (unusual) / u - u a l (usual)

Comprehension question: Do you think your score is lower than expected on this test?

54) Scenario: You decide to attend an exam preparation class for the course you are studying. The presenter explains that because the test has been arranged to provide questions to suite a range of ability, in order to pass, students in this class would need to correctly answer a f - - (few) / l - - (lot)

Comprehension question: Do you need to answer most questions correctly to pass?

55) Scenario: Your boss asks you to do a job at work. You finish it before the deadline, although he finds some mistakes in it. You are new to the job and feel that your boss will think you are p - - g r - s s - n g (progressing) / n e g - - g e - t (negligent)

Comprehension question: Is your boss satisfied with you?

56) Scenario: You are asked to attend for an interview procedure at a recruitment centre with many others. You complete several written tests and receive feedback. You got very few questions correct and you answered the most important items c - - r e c t - - (correctly) / i m - r - p - r l y (improperly)

Comprehension question: Do you think your performance was good enough on the important questions?

57) Scenario: You are playing a game of cricket with friends and it is your turn to bat. You manage to hit a few balls before you are out. You think that the shots you missed were a result of overall poor b o - l - n g (bowling) / p e r - o r - a n c e (performance)
Comprehension question: Did you blame yourself for not doing well at cricket?

58) Scenario: You are taking a music exam and have to do a sight-reading test. As you try and play you realise that it is not easy and you make some mistakes. After you finish the examiner tells you that the level of difficulty of the piece you were asked to play was deliberately chosen to be d i - - i c u – t (difficult) / e a - - (easy)
Comprehension question: Were you expected to play well?

59) Scenario: You are short-listed for a job that you really want and after an interview you are asked to take some aptitude tests. Afterwards you are given feedback on your answers and are told they are nearly all wrong. You guess that they were testing your reactions to stress when you are later told you have been s u - - e s - f – l (successful) /
u n s u - - e s - f u l (unsuccessful)
Comprehension question: Did you handle the stressful interview acceptably?

60) Scenario: You are meeting an old friend that you haven't seen for years. As you think of how your life has progressed since you last saw them you wonder if they will think that compared with them your ups and downs add up to a life that has been quite
f - l f - l l i – g (fulfilling) / u n i n - e r - s t - - g (uninteresting)
Comprehension question: Do you think your friend will think you have had a good life?

61) Scenario: As a member of the fundraising team at a local school, you are asked to organise a bazaar. You do your best although there is little time and you don't think that you have done a very good job. When you get feedback you hear that compared to last year it was s u - e r - - r (superior)/ w - - s e (worse)
Comprehension question: Did people prefer last year’s bazaar?

62) Scenario: You are persuaded to join a quiz team in a tournament. You are told that most of the questions will be asked to individuals in specialist rounds. The first game is hard and you don’t get many of your questions right. Afterwards you hear the others talking about you, they are saying that compared to them they think you did o - - y (okay) /
b - d - y (badly)
Comprehension question: Are the other members of the team pleased with how you performed?

63) Scenario: You enrol on a course to learn to administer intelligence tests. Working through some items in an example test you find that after the first few examples you cannot solve

any more questions. Later on you are handed a manual for the test and look up what your score indicates. Your final score is listed as showing an ability level that is
h - - h (high) / l - - (low)
Comprehension question: Does the task indicate that your intelligence is low?

64) Scenario: You have a go at doing the mental puzzles in your newspaper and find them surprisingly difficult. You are surprised because you think that you have very many good qualities and think that being good at solving this sort of puzzle is relatively
u n i - p o - t - - t (unimportant) / i - p o - t - n t (important)
Comprehension question: Does it bother you that you are finding the puzzles difficult?

65) Scenario: You decide to sign up for a residential 'learning for fun' course. You choose woodworking and first of all everyone is given a test to reveal their existing expertise. You cannot do many of the tasks set so you are allocated to a group with others who are less able. You notice how the other groups progress and conclude your group is finding it more f - - (fun) / t o u - - (tough)
Comprehension question: Is your group enjoying the week?

66) Scenario: You try to help your friend's son with GCSE maths. You can't answer any of the questions and think back to how you did when you took the exams. Quickly you realise that you cannot help because the skills you had were quite d i f - e r - - t (different) / w - - k (weak)
Comprehension question: Are the skills you have too feeble?

67) Scenario: You decide to have a go at some online crosswords. Quickly you realise that you are not very good and cannot work out any of the clues, just as you decide to stop playing you see that the ability level was set to e x - - r t (expert) / n o - - c e (novice)
Comprehension question: Are you concerned that you cannot solve any of the clues?

68) Scenario: In quite a long task you are required to attempt a number of items but many of them seem impossible. When you finish you are told that the session was designed to test a particular attribute. The items are deliberately difficultbut to achieve well on this attribute, you need to show evidence of mental r - s i l - e - c e (resilience) /
i m - r o v - m e n t (improvement)
Comprehension question: Was this a test of your determination?

69) Scenario: Your friend is very keen on skating and persuades you to try it out. At the rink you put on the skates and step on the ice. You glide forward, slowly at first, then faster, your feet don't seem to obey you instructions. As you continue you start to feel e - c i t – d (excited) / d - z z - (dizzy)

Comprehension question: Do you feel well?

70) Scenario: You are on a long journey and suddenly traffic comes to a halt. The road is closed and you have to make a detour. After only a few minutes you realize you must have made a wrong turn and there are no more diversion signs. You find yourself winding through a maze of small roads concluding that taking this route has turned out to be very b - - u - i f – l (beautiful) / s t r - s s - - l (stressful)
Comprehension question: Did you enjoy driving through country roads?

Neutral Scenarios (embedded into positive training)

1) Scenario: An acquaintance calls to ask you for some advice about a relationship problem. The conversation soon drifts onto other things and before you realise the time, you find that you have spent most of the afternoon t - - k i n – (talking)
Comprehension question: Did you speak with your friend in the evening?
2) Scenario: You and a friend decide to join an evening class in pottery. When you arrive on the first night, you discover that the class is held in a converted barn. Because it was chilly outside you think you should have brought a s w - a t e – (sweater)
Comprehension question: Is the class held in a converted barn?
3) Scenario: You arrange to visit a friend who lives some distance away and plan to travel by coach. When you get on, the coach is fairly empty and so you take a double seat at the front. After several hours of travel you start to feel s - e - p y (sleepy)
Comprehension question: Did you sit in the back of the bus on your trip?
4) Scenario: A friend calls you up to suggest that you meet up for dinner one evening. You arrive at the restaurant and are quickly seated. The waiter hands you a menu and as you read it you notice that you are feeling quite h - n g - y (hungry)
Comprehension question: Were you eating with a friend?
5) Scenario: One day at work, your boss rings through and tells you that a colleague is retiring at the end of the month. As this was someone that everyone knew very well, he suggests that you could get together with others in your office and organise a p - - t y (party)
Comprehension question: Is one of your work colleagues leaving soon?
6) Scenario: You decide to take up jogging and plan to go out every morning before work. On the first morning, you get up early and put on your tracksuit and then head off for your first run. You decide to start by alternating short bursts of running with some

w a - k - n g (walking)

Comprehension question: Did you go for a jog after work?

7) Scenario: You finish work early so that you can go to a local firm of solicitors to collect some papers for your boss. When you get to the offices, you report to reception. You explain to the secretary who you are. She asks you to take a seat in the l - - n g e (lounge)
Comprehension question: Did you speak to the secretary before you collected the papers?
8) Scenario: You attend a schooldays reunion at your old college and meet up with lots of people you have not seen for some time. You speak to lots of old friends and then decide to get a drink. You go to the bar and when you return you find that some of your friends are dancing to loud m u - i c (music)
Comprehension question: Was the music quiet?
9) Scenario: You inherit an old dining table and chairs and decide to restore them to their former glory. You spend hours in the garage working on them. When you have finished working on them, you bring them into the h o u - - (house)
Comprehension question: Did you buy and old dining set?
10) Scenario: It is almost time for your town's spring festival. A friend of yours is on the committee and asks if you would be prepared to help out with the barbecue in the park. You hope that on that day it will be s u - - y (sunny)
Comprehension question: Does your town hold the festival in spring?
11) Scenario: It is your partner's birthday soon and you want to do something special. You put a lot of thought into what to do and after much discussion, you decide on a weekend break to Paris. You book the tickets online immediately, and print out the c o - f - r m a t - o n (confirmation)
Comprehension question: Will you be travelling for a week long holiday on your partner's birthday?
12) Scenario: Your firm organises a bowling trip as an annual social event. Because you are a new employee and have not been at this firm for very long this will be your first trip. Every year the trip is popular and because many people were going everyone travelled by c o a - - (coach)
Comprehension question: Did your firm organise a bowling trip?
13) Scenario: Your new neighbours have just moved in last week. Your neighbours ask if you would like to go round for a drink the following evening. When you arrive, there are other people there who you do not know and you soon find yourself being i - t r - d - c – d (introduced)

Comprehension question: Did you have to wait for others to arrive?

14) Scenario: Last year you decided you wanted to take up another hobby. You became a member of a hockey club and now regularly play for the second team on Saturday afternoons. One week, the captain tells you that there is to be an extra midweek m a - c h (match)

Comprehension question: Do you play hockey on the second team?

APPENDIX VI – Study 5: Interpretive Bias Test Stimuli

1) Category: Test

   Title: The Fundraising Quiz

   Scenario: You are attending a fundraising quiz evening with lots of teams taking part. In one round you have some unusual puzzles to complete. Your team is having little success and as you look around to try and see how rival teams are doing you think you can tell what the relative scores for this round will b – (be)

   Comprehension question: Do you have to complete some unusual puzzles?

   Positive target: You look round and see that no-one else can do any of the puzzles

   Negative target: You look round and see that everyone else can do the puzzles

   Positive foil: You look round and contemplate what an enjoyable event this is

   Negative foil: You look round and see people from the other teams are looking back at you crossly

2) Category: Test

   Title: The Ten Minute Warning

   Scenario: The warning is given that there are ten minutes left before the end of a long and draining three hour exam. As you check through your answers you think that the exam has been a lot harder than you had expected. You then hand your paper in and reflect on how you think you did in comparison to the other s t - d - n t – (students)

   Comprehension question: Were you in a one hour exam?

   Positive target: You hand your paper in and think that you did well in comparison to other students

   Negative target: You hand your paper in and think that you did badly in comparison to other students

   Positive foil: You hand your paper in and feel pleased that you have finished all your exams

   Negative foil: You hand your paper in and you were disappointed that you didn't finish in time

3) Category: Social

   Title: The joke

   Scenario: You are with a group of new friends, on your way to an open air concert. You decide to tell a joke you heard recently. Everyone looks at you as you start telling the joke, and you see their expressions change when you get to the punch l - n e (line)

Comprehension question: Did you hear the joke you told quite recently?

Positive target: When you get to the end you see everyone starting to laugh

Negative target: When you get to the punch line everyone looks confused

Positive foil: When you get to the end you receive several compliments

Negative foil: When you start telling your joke someone interrupts you

4) Category: Social

Title: The private view

Scenario: Your neighbour invites you to a private exhibition of his art. You arrive to find many other guests whom you do not know. You try talking to some of them, and can see how interested they are in your c - - v e r - - t i – n (conversation)

Comprehension question: Was the private view at a relative's house?

Positive target: You talk to some guests and can tell that they find you interesting

Negative target: You talk to some guests but they think what you say is boring

Positive foil: You meet many guests whom you know and enjoy talking to them

Negative foil: You don't know anyone there and everyone ignores you completely

5) Category: Test

Title: The Bioengineering Exam

Scenario: You have chosen a difficult subject for one of your options. You have done some coursework but 50% of your assessment is an exam with a series of short answers, chemical formulae and definitions. There are lots of questions and so you go through as fast as you can to find some you can answer. You've been told the pass mark for the exam so by the end think you can guess how well you have d - - e (done)

Comprehension question: Have you chosen an easy subject for one of your options?

Positive target: After the exam you think you have enough correct answers to pass

Negative target: After the exam you think you do not have enough correct answers to pass

Positive foil: After the exam you know your high coursework mark already ensures a pass

Negative foil: After the exam you know your poor coursework already means that you will fail

6) Category: Social

Title: Your birthday

Scenario: It is your birthday and you wake up looking forward to your day. You wonder how many friends will send you a birthday card. However, you have to go to work as usual, and by the time you leave, no cards have a r r - v – d (arrived)

Comprehension question: Did you have to go to work on your birthday?

Positive target: You have to leave for work before the postman brings your mail

Negative target: You leave for work realising that no one has sent you a card

Positive foil: You leave for work feeling pleased with the cards you have received

Negative foil: You leave for work knowing that it is going to be a stressful day

7) Category: Social

Title: Meeting a friend

Scenario: In the street, you bump into an old friend you haven't seen for a long time. She is too busy to stop, so you arrange to meet later in a bar. You arrive a little late but the bar is empty and a few minutes later she is still not t h - - e (there)

Comprehension question: Was anyone else in the bar?

Positive target: You arrange to meet a friend in a bar but your friend is late

Negative target: You arrange to meet in a bar but your friend stands you up

Positive foil: You are busy but your friend insists on meeting you in a bar

Negative foil: Your friend tells you that she does not want to meet you

8) Category: Test

Title: The Spanish Exam

Scenario: You are learning Spanish and are encouraged to take an exam to see how you do. The exam is quite difficult and you don't understand a lot of the questions so cannot answer them. Thinking back to the few questions that you did manage to answer you review your progress so f – r (far)

Comprehension question: Are you learning Italian?

Positive target: The think that your progress is sufficient to pass the Spanish exam

Negative target: You think that your progress is not sufficient to pass the Spanish exam

Positive foil: You think that you are glad that you decided to start learning Spanish

Negative foil: You think that starting to learn Spanish was probably a bad idea

9) Category: Social

Title: The job interview

Scenario: You applied for a job in a company you'd really like to work in. You are invited to an interview, where you answer the questions as well as you can. Reflecting later, you think that the quality of your answers decided the o u - c o m – (outcome)

Comprehension question: Did you think about your answers later?

Positive target: You think that your astute answers led to you being offered the job

Negative target: You think that your poor answers lost you the job

Positive foil: You think it was a good thing you did not take the job

Negative foil: You think your poor reference must have made a bad impression

10) Category: Social

Title: The bus ride

Scenario: You get on a bus and find an empty seat next to one that has a rip in it. At the next stop several people get on that you vaguely recognise, but they sit together and the seat next to you remains v - c a – t (vacant)

Comprehension question: Were the people who got on strangers to you?

Positive target: The seat next to you remains empty because it looks damaged

Negative target: The seat next to you is empty because no one wants to sit with you

Positive foil: The person in the seat next to you talks to you in a friendly way

Negative foil: The person in the seat next to you makes a rip in the fabric

11) Category: Test

Title: The Intelligence Test

Scenario: You browse through a book in the library which has some examples of questions set to join a new society for those with above average intelligence. You try a few but they are really hard and your success rate is very low. As you already know your own intelligence level you think about the accuracy with which this test estimates i n t - l l - g - - c e (intelligence)

Comprehension question: Are you in a library?

Positive target: You think that the test does not estimate intelligence very well

Negative target: You think that the test estimates intelligence well

Positive foil: You think that the test is quite enjoyable to complete

Negative foil: You think that the test is quite irritating to complete

12) Category: Test

Title: The Challenge at Work

Scenario: You are set a really difficult challenge at work. You are really determined and so you persevere with the work even though you are finding it almost impossible. When you go home that night you reflect on what your boss probably thinks of your a b - l - - y (ability)

Comprehension question: Are you set a challenge at college?

Positive target: You reflect that your boss probably thinks you are a hard-working employee

Negative target: You reflect that your boss probably thinks you are not very good at your job

Positive foil: You reflect that you are lucky to have such a nice boss

Negative foil: You reflect that your boss probably finds your personality disagreeable test

13) Category: Social

Title: Your first painting

Scenario: You've taken up painting as a hobby, and have just finished your first picture. You hang it on the wall when a group of friends visit. Later you overhear your friends making remarks that make clear their opinion of your t - l - n t (talent)

Comprehension question: Did you leave the painting on an easel?

Positive target: You overhear some friends saying how much they liked your painting

Negative target: You overhear some friends making critical remarks about your picture

Positive foil: You overhear some complimentary remarks about your good taste

Negative foil: You overhear some friends making fun of something you just said

14) Category: Test

Title: The Job Interview

Scenario: You have passed the interview stage of a new job but now must do a battery of tests with others applying for different posts with this company. You progress through memory, logic and maths problems, some easy and then some very difficult versions. At the end you can tell you did not get many correct on some tasks. As you leave you reflect on how you think you performed on the most relevant q u - s t - - n s (questions)

Comprehension question: Do you have to do a battery of tests?

Positive target: You reflect that you probably performed well on the most relevant questions

Negative target: You reflect that you probably performed poorly on the most relevant questions

Positive foil: You reflect that the interviewers made encouraging comments as you left the room

Negative foil: You reflect that the interviewers made discouraging comments as you left the room

15) Category: Test

Title: University Challenge

Scenario: You have responded to an advert to join the team for "University Challenge". To select members you all come together and try to answer a series of questions. As you work through them you struggle with some, definitely get some correct and are unsure

about others. Judging by the answers of the others and the expressions of the selectors you have a good idea who will be c - o s – n (chosen)

Comprehension question: Did you respond to an advert for "Mastermind"?

Positive target: The selectors make their choice and you are included in the team

Negative target: The selectors make their choice and you are excluded from the team

Positive foil: The selectors make their choice and there is happy chatter as the team is announced

Negative foil: The selectors make their choice and there is a depressed air as the team is announced

16) Category: Test

Title: The Computer Class

Scenario: You are taking a computer class that involves a test. As you work through the test items you have been set you find them hard to answer and give up on many of them. You worry if this reflects on your lack of ability. Talking later to the other people who took the same test you find out how they all d – d (did)

Comprehension question: Are you taking a computer class?

Positive target: You find out that other people found the test very difficult

Negative target: You find out that other people found the test quite easy

Positive foil: You find out that you are invited to a sociable get-together after the test

Negative foil: You find out that no-one wants to talk to you after the test has finished

17) Category: Social

Title: The first aid refresher

Scenario: You participate on a first aid refresher course at work. The instructor asks a question and no one in the group volunteers an answer, so he looks directly at you. You offer a reply, thinking about how your answer must be sounding to the o t h - - s (others)

Comprehension question: Was the refresher course organized by a local charity?

Positive target: You answer the question, thinking that the others may be quite impressed

Negative target: You answer the question, thinking how ignorant you may seem

Positive foil: You answer the question, pleased that you have such an interesting teacher

Negative foil: You answer the question, realising you are irritated by this teaching style

18) Category: Social

Title: The local club

Scenario: You are invited for a night out at a local club, although you don't know any of the members very well. As you approach the door you can hear loud music and noisy conversation, but as you enter the room it is quiet for a m - m - n t (moment)

Comprehension question: Do you know most of the club members?

Positive target: As you enter the room the music stops for a moment

Negative target: As you enter the room everyone stops and stares at you

Positive foil: As you enter the room someone greets you warmly

Negative foil: As you enter the room someone asks you why you are there

19) Category: Test

Title: The Practical Driving Test

Scenario: While taking a driving test you make an error when parking. You continue with the other manoeuvres and consider how much credit the examiner will give for your good driving in the rest of the test. At the end of the test you are told the outcome which is much as you p r e - - c t – d (predicted)

Comprehension question: Did you continue with the other manoeuvres?

Positive target: You finish the driving test and the examiner says you have passed

Negative target: You finish the driving test and the examiner says you have failed

Positive foil: You finish the driving test and the examiner praises your driving skills

Negative foil: You finish the driving test and the examiner is rude about your driving ability

20) Category: Social

Title: The wedding reception

Scenario: Your friend asks you to give a speech at her wedding reception. You prepare some remarks and when the time comes, get to your feet. As you speak, you notice some people in the audience start to l - - g h (laugh)

Comprehension question: Did you stand up to speak?

Positive target: As you speak, people in the audience laugh appreciatively

Negative target: As you speak, people in the audience find your efforts laughable

Positive foil: As you speak, people in the audience applaud your comments

Negative foil: As you speak, some people in the audience start to yawn in boredom

APPENDIX VII – Study 6A: CBM-A Stimuli

Table 29

CBM-A word pairs, list 1

Word Pair	Emotional word	Neutral word	Emotional word category
1	unpopular	countless	general
2	mistaken	theology	general
3	squeamish	lamplight	sensation
4	jittery	pervade	sensation
5	anguish	jackets	sensation
6	dizzy	foyer	sensation
7	excluded	imperial	general
8	shiver	pearls	sensation
9	uptight	doorman	general
10	useless	lending	general
11	shudder	coconut	sensation
12	distraught	camouflage	sensation

Table 30

CBM-A word pairs, list 2

Word Pair	Emotional word	Neutral word	Emotional word category
1	nervous	painted	sensation
2	vomiting	infinity	sensation
3	boring	employ	general
4	tremor	dusted	sensation
5	imperfect	resonance	general
6	shunned	oratory	general
7	pathetic	loading	general
8	upset	cycle	sensation
9	pain	mass	sensation
10	scorned	revolve	general
11	frail	Poses	sensation
12	lightheaded	exfoliating	sensation

Table 31

CBM-A word pairs, list 3

Word Pair	Emotional word	Neutral word	Emotional word category
1	unsettled	facsimile	sensation
2	shaking	estates	sensation
3	embarrassed	transmission	sensation
4	coward	piping	general
5	sweating	allocate	sensation
6	intimidated	discounting	sensation
7	selfish	lorries	general
8	stupid	occurs	general
9	unfriendly	immaterial	general
10	inferior	advocate	general
11	restless	rigorous	sensation
12	alone	stood	sensation

Table 32

CBM-A word pairs, list 4

Word Pair	Emotional word	Neutral word	Emotional word category
1	negligent	certified	general
2	unstable	surveyor	general
3	flustered	veritable	sensation
4	twitchy	coolant	sensation
5	mocked	orbits	general
6	tremble	rallied	sensation
7	panic	canal	sensation
8	inadequate	electrical	general
9	shame	craft	sensation
10	faint	habits	sensation
11	abandoned	conducted	general
12	overwrought	divergences	general

APPENDIX VIII – Study 6B: CBM-I Stimuli

Table 33

CBM-I association words, list 1

Homograph	**Negative Fragment Resolution**				**Neutral Fragment Resolution**			
Prime	***1***	***2***	***3***	***4***	***1***	***2***	***3***	***4***
Batter	bruise	mistreat	abuse	violence	pudding	pancake	fried	fish
Beat	slapping	smash	strike	impact	rhythm	accent	tempo	drumming
Blow	misfortune	setback	calamity	trauma	respire	puff	inhale	exhale
Execute	electrocute	behead	hanged	shoot	accomplish	achieve	effect	complete
Hit	smite	slap	concussion	whack	popular	success	favourite	winner
Institution	psychiatric	hysterical	deluded	psychotic	traditional	wedding	ritual	marriage
Lie	cheat	untrue	deceive	truth	relax	recline	laze	mattress
Mean	unkind	horrible	stingy	cruel	propose	expect	intention	intend
Ram	collide	crash	accident	barge	ewes	paddock	horns	sheep
Rattle	fluster	unnerve	disturb	confuse	shake	noisy	jangle	instrument
Scan	diagnosis	clinic	medical	hospital	peruse	scrutinise	survey	study
Sharp	blade	razor	pointed	stab	acute	shrewd	keen	clever
Shot	killed	gunned	blast	wounded	whisky	beverage	vodka	spirits
Stalk	pursue	stealth	hunted	creep	flower	plant	leafy	branch
State	plight	situation	panic	predicament	affirm	pronounce	declare	assert
Wound	laceration	flesh	lesion	suture	tight	wrapped	twine	bundle

Table 34
CBM-I association words, list 2

Homograph	**Negative Fragment Resolution**				**Neutral Fragment Resolution**			
Prime	***1***	***2***	***3***	***4***	***1***	***2***	***3***	***4***
Abort	unborn	babies	foetus	pregnant	schedule	timetable	cancel	agenda
Brood	mope	obsess	sulk	worry	chicks	litter	eggs	newborn
Capital	corporal	punishment	hanged	offense	expenditure	investment	assets	income
Chill	frightening	fearsome	afraid	terror	brisk	freezing	frost	wintry
Crank	eccentric	misfit	weirdo	oddball	engine	shaft	wheel	axle
Fit	epileptic	spasm	seizure	uncontrollable	athletic	exercise	well	strong
Frame	innocent	implicate	incriminate	evidence	border	casing	enclose	outline
Growth	lump	radiation	tumour	malignant	height	enlarge	shrink	increase
Incense	aggravate	fury	inflame	provoke	smell	fragrance	scent	odour
Parting	farewell	separating	going	leaving	haircut	brushing	hair	hairstyle
Quiver	shudder	timid	scared	palpitate	archer	quill	bow	bowman
Row	debate	fight	argument	shouting	column	houses	queue	series
Sack	retrench	employment	unemployed	redundant	container	bag	cloth	carry
Terminal	untreatable	illness	virus	disease	aeroplane	airport	depot	station
Wake	ritual	death	respects	vigil	yawn	arise	asleep	morning
Wrench	anguish	dislocated	strain	ankle	spanner	screwdriver	mechanic	unbolt

Table 35
CBM-I association words, list 3

Homograph	**Negative Fragment Resolution**				**Neutral Fragment Resolution**			
Prime	***1***	***2***	***3***	***4***	***1***	***2***	***3***	***4***
Arresting	apprehend	seize	capture	catch	gorgeous	stunning	spectacular	attractive
Bitter	vicious	resentful	hostile	spiteful	vinegary	flavour	acrid	tart
Charge	accusation	blame	allegation	indictment	price	credit	expensive	account
Chop	knife	whacked	severed	hacked	barbeque	butcher	steak	hamburger
Committed	asylum	uncontrollable	breakdown	psychiatrist	pledged	dedicated	worker	engaged
Cross	complaining	fuming	annoyed	vexed	church	priest	holy	religion
Crush	massacre	crumble	destroy	defeat	strawberry	blend	squash	pineapple
Gag	hostage	bonds	muffle	muzzle	prank	laugh	funny	witty
Hamper	restrict	frustrate	impede	obstruct	picnic	basket	christmas	sandwiches
Hang	neck	noose	strangle	suicide	raincoat	jacket	coat	clothing
Low	unfair	underhanded	backstabbing	sneaky	tiny	squat	small	short
Punch	clout	knuckles	boxing	knock	refreshment	beverage	liquor	juice
Shaken	frightened	tremulous	upset	agitated	whirl	cocktail	martini	stirred
Tramp	pauper	homeless	vagrant	despondent	trundle	walk	stamp	trudge
Undertaking	mortuary	burial	coffin	funeral	enterprise	venture	endeavour	mission
Will	inheritance	solicitor	testament	testimony	want	power	determination	purpose

Table 36
CBM-I association words, list 4

Homograph	**Negative Fragment Resolution**				**Neutral Fragment Resolution**			
Prime	***1***	***2***	***3***	***4***	***1***	***2***	***3***	***4***
Badger	bother	pester	torment	irritate	mole	bat	fox	burrow
Bark	hound	growl	dog	yapping	plants	foliage	undergrowth	tree
Bit	chew	teeth	tooth	snap	fragment	little	piece	crumb
Block	obstacle	deter	obstruction	prevent	cement	concrete	brick	metal
Body	cadaver	carcass	corpse	remains	shape	physique	person	form
Cane	headmaster	smack	teacher	school	stem	wicker	furniture	fronds
Decline	frailty	sickness	degeneration	senility	invitation	refuse	offer	accept
Die	expire	perish	deceased	demise	gamble	game	cast	numbers
Fine	legal	payment	infringement	parking	miniscule	small	grain	granular
Nuts	madman	mental	unbalanced	crazy	peanuts	almonds	brazil	hazel
Plot	against	secretive	connive	conspire	scenario	storyline	narrative	story
Pound	injure	wallop	pulverize	thump	ounce	kilogram	heavy	scales
Ruin	career	bankrupt	reputation	impoverish	ancient	archaeology	castle	historical
Shady	character	dishonest	dodgy	suspicious	cloudy	sunny	cool	dark
Sink	immersed	float	ferry	ship	washing	kitchen	bathroom	dishes
Sour	displeased	unpleasant	dissatisfied	unhappy	oranges	fruit	taste	lemons

Table 37
CBM-I association words, list 5

Homograph	**Negative Fragment Resolution**				**Neutral Fragment Resolution**			
Prime	***1***	***2***	***3***	***4***	***1***	***2***	***3***	***4***
Bars	restrain	criminal	chains	shackles	beers	tavern	drinks	alcohol
Booking	police	arrest	fined	warden	theatre	cinema	reservation	seats
Bound	captive	confined	hostage	tight	gallop	bounce	skip	sprint
Box	biff	match	injured	scuffle	lid	canister	storage	case
Cell	jail	warden	guard	prison	biology	amoeba	germ	microscopic
Critical	dangerous	condition	dying	deathly	important	essential	crucial	key
Dressing	treatment	gauze	hospital	bandage	clothed	fashion	robing	gown
Graze	scrape	blood	scratch	knee	horses	cattle	meadow	cows
Infectious	contagious	epidemic	plague	influenza	laughter	enjoyment	merriment	enthusiasm
Maroon	stranded	shipwreck	isolated	helpless	purple	brown	colour	reddish
Mug	attack	thief	robbery	assail	tankard	flagon	teacup	vessel
Rank	repellent	putrid	offensive	repulsive	soldier	corporal	general	military
Revolution	overthrow	communism	insurrection	anarchy	gyration	revolving	turning	rotation
Slice	slash	dissect	cleave	impale	turkey	plateful	portion	bowlful
Stern	tough	firm	frown	harsh	cruise	captain	galley	yacht
Twisted	deranged	perverted	cruel	strange	string	contorted	round	coiled

Table 38
CBM-I association words, list 6

Homograph	**Negative Fragment Resolution**				**Neutral Fragment Resolution**			
Prime	***1***	***2***	***3***	***4***	***1***	***2***	***3***	***4***
Appeal	verdict	plead	lawful	conviction	likeable	enticing	intense	sexual
Arms	guns	pistols	shotguns	rifles	legs	stretch	length	shoulders
Bind	problem	perplexity	crisis	dilemma	connect	fasten	attach	tie
Blind	sight	eyes	spectacles	seeing	cover	shade	window	curtain
Cracked	demented	madness	paranoid	insane	china	ceramic	porcelain	saucer
Late	morgue	cremation	grieve	buried	punctual	early	arrival	delayed
Mine	ammunition	explode	warhead	fuse	belonging	yours	possession	ours
Odd	weird	bizarre	peculiar	unusual	amount	uneven	digit	integer
Petrified	horrified	fearful	freaked	aghast	hardened	solidified	fossilised	granite
Shell	mortar	cannon	missile	grenade	beach	oyster	fishes	seaside
Strained	worry	headache	angst	anxiety	separate	drain	sifter	sieved
Striking	hitting	force	cuffing	pummelling	resemblance	pretty	extraordinary	dazzling
Stump	hobble	mutilate	maimed	cripple	oaken	lumber	timber	root
Temper	outrage	tantrum	furore	annoy	soothe	moderate	mitigate	soften
Tense	stressed	relaxed	nervous	anxious	future	past	present	grammar
Vault	tomb	underground	cavern	chamber	jump	hurdle	upwards	launch