Daily Emotional Functioning in Social Anxiety Disorder

by

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Abstract

Social Anxiety Disorder (SAD) is characterised by fear or anxiety around potential judgement, scrutiny and negative evaluation by others in social situations. For those with the disorder, social engagement can lead to considerable distress and functional impairment in daily life. Therefore, how individuals with SAD respond to stress, specifically social stress, is of particular importance to the understanding and treatment of the disorder. Much of the existing SAD research has been conducted in the laboratory setting, which provides optimal experimental control but offers little insight into how the disorder plays out in daily life. The symptoms of SAD are context-dependent and fluctuate over time, making them difficult to assess realistically in the laboratory or using retrospective reporting. Ambulatory assessment could deepen our understanding of the symptoms and experiences of those with SAD through frequent assessments in their naturally occurring environment. However, it is difficult to capture how individuals with SAD respond to social stressors using a traditional ambulatory assessment design, as SAD is associated with avoidance of such situations in daily life. This thesis examined the acute social stress response of those with SAD in daily life.

A standardised lab-induced social stressor was embedded within an ambulatory assessment design to study the effect of acute social stress on naturalistic subjective and physiological stress responding among individuals with SAD (n = 40) and healthy controls (n = 41). After completing two days of *baseline* daily life assessment, participants were informed that they would complete a social stress task (the Trier Social Stress Test; TSST) in two days' time. Following the TSST, participants continued with daily life assessment for an additional two days. This distinguished the *anticipatory* (days prior to TSST), acute (during the TSST protocol) and *recovery* (days after TSST) phases of stress responding. Subjective responses were assessed using a smartphone app called SEMA and physiological responses

were collected on three days (once during each phase) through ambulatory saliva sampling.

The first empirical study of this thesis (Study 1, Chapter 6) reports on the acute social stress response to the TSST assessed in the lab, compared between individuals with SAD versus healthy controls. The second large scale empirical study (Study 2, Chapter 7) reports the results of naturalistic responding to the TSST in daily life, captured using ambulatory assessment, in the same participants. Results from the two empirical studies demonstrated that overall individuals with SAD reported a significantly worse experience across all measures of affect, self-esteem and threat-awareness when compared to healthy controls. Between group comparison during the anticipation of social stress in daily life found those with SAD responded with increased anxiety, reduced happiness and less appearance satisfaction, when compared to healthy controls and baseline. In response to social stress, SAD individuals responded with increased stress sensitivity in their subjective experience in the lab and outside of the lab in daily life, seen in the increased anxiety and anger, reduced happiness and less appearance satisfaction reported during the recovery from a social stressor, compared to healthy controls. However, between group comparison revealed no physiological (salivary cortisol) differences were observed between SAD and healthy controls in either the lab or daily life settings.

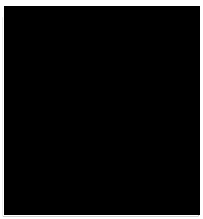
Overall, this thesis adds novel information to the understanding of SAD, especially to the subjective and physiological experience of SAD in daily life in response to social stress. This thesis supports models of SAD that highlight cognitive, psychological and behavioural factors in the aetiology and maintenance of the disorder. Lastly, this thesis provides a valuable source in the form of a laboratory manual (see Chapter 5) to ease the application of implementing the TSST by other researchers.

General Declaration

This thesis contains no material that has been extracted in whole or in part from a thesis that I have submitted towards the award of any other degree or diploma in any other tertiary institution.

No other person's work has been used without due acknowledgment in the main text of the thesis.

All research procedures reported in the thesis received the approval of the relevant Ethics/Safety Committees (where required).



Signed by Caitlin Grace on January 6th, 2020

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Abbreviations and Symbols

α – alpha

χ^2 – Chi-squared

η^2 – Eta-squared

µg/dL – Micrograms per Deciliter

% – Percent

AA – Ambulatory Assessment

ACTH – Adrenocorticotropic Hormone

ACU – Australian Catholic University

ADAVIC – Anxiety Disorders Australian Victoria

ANS – Autonomic Nervous System

APA – American Psychiatric Association

ASQ – Attachment Style Questionnaire

AUC – Area Under the Curve

AUCg – AUC with respect to ground

AUCi – AUC with respect to increase

BF_{10} – Bayes Factor

BFI – Big Five Inventory

BFNE – Brief Fear of Negative Evaluation Scale

CAR – Cortisol Awakening Response

CBGT – Cognitive Behavioural Group Therapy

CBT – Cognitive Behavioural Therapy

CI – Confidence Interval

CIDI – Composite International Diagnostic Interview

CNS – Central Nervous System

CRH – Corticotropin-Releasing Hormone

CrI – Credible Interval

CTQ – Childhood Trauma Questionnaire

d – Cohen's *d*

DASS – Depression Anxiety Stress Scale

df – Degrees of Freedom

DSM – Diagnostic and Statistical Manual of Mental Disorders

ECG – Electrocardiogram

EMA – Ecological Momentary Assessment

FE – Fisher's Exact Test

FPES – Fear of Positive Evaluation Scale

g – Centrifugal Force

GABA – Gamma Amino Butyric Acid

GAD – Generalised Anxiety Disorder

GPS – Global Positioning System

H – Kruskal-Wallis H test

H_0 – Null Hypothesis

H_1 – Alternate Hypothesis

HC – Healthy Control

HPA – Hypothalamic Pituitary Adrenal (axis)

HREC – Human Research Ethics Committee

IAM – Integrated Aetiological and Maintenance

ICC – Intraclass Correlation Coefficient

ICD – International Classification of Disease

LMEM – Linear Mixed Effects Model

LSAS – Liebowitz Social Anxiety Scale

M – Mean

MAOI – Irreversible Monoamine Oxidase Inhibitor

MDD – Major Depressive Disorder

mg – Milligram

min - Minutes

MINI – Mini International Neuropsychiatric Interview

ML – Maximum Likelihood

MRI – Magnetic Resonance Imaging

N – population size

n – sample size

nmol/L – Nanomoles per liter

NR – Salivary Cortisol Non-Responders

OCD – Obsessive-compulsive Disorder

p – Probability value; significance

pg/ml - Picogram/milliliter

PTSD – Post Traumatic Stress Disorder

r – Cohen's *r*

R – Salivary Cortisol Responders

RCT – Randomised Control Trial

RIMA – Reversible Inhibitor of Mono-Amine Oxidase A

RSE – Rosenberg Self-Esteem Scale

SAD – Social Anxiety Disorder

SAM – Sympathetic Adrenal Medullary

SCID – Structured Diagnostic Interview for the DSM

SD – Standard Deviation

SE – Standard Error

SEMA – Smartphone Ecological Momentary Assessment

SET – Social Evaluative Threat

SIAS – Social Interaction Anxiety Scale

SNRI – Serotonin and Norepinephrine Reuptake Inhibitors

SOS – SalivaBio Oral Swab

SPSS - Statistical Package for the Social Sciences

SSRI – Selective Serotonin Reuptake Inhibitors

STAI – State-Trait Anxiety Inventory

t – *t*-test statistic

TSST – Trier Social Stress Test

U – Mann-Whitney U test statistic

VAMS – Visual Analogue Mood Scale

W – Bayesian Mann-Whitney U Test Statistic

z – z statistic

CHAPTER 1. General Introduction and Thesis Outline

1.1. Introduction

Human beings are naturally social creatures. Take a moment to reflect back across your past week and consider how many activities you engaged in that involved other people. Perhaps you went out for coffee with a friend, went on a date, worked in an office, went to the supermarket, ate out at a restaurant, or went to the gym. While these events are commonplace and seemingly innocuous for most people, for some individuals, these situations provoke a significant amount of fear and anxiety. This fear and anxiety stems from concerns related to potential judgement, scrutiny, and negative evaluation by others, and are experienced by some to such a degree that they reach clinical levels of distress. Such individuals then meet the diagnostic criteria for Social Anxiety Disorder (SAD).

Social Anxiety Disorder is characterised by clinically significant fear and anxiety toward the potential judgement, scrutiny, and negative evaluation by others during social engagement (American Psychiatric Association, 2013). It was first introduced as a diagnostic category in the third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM - III; American Psychiatric Association, 1980) and initially viewed as a minimally intrusive or disruptive disorder. However, the breadth of research that now exists investigating SAD has revealed that the disorder largely impairs functioning and can lead to high levels of distress and have a major impact on an individual's quality of life (Heimberg & Magee, 2014). SAD has been linked to significant distress and impairment in functioning across multiple domains including occupational and academic functioning, and interpersonal relationships (American Psychiatric Association, 2013). The excessive fear and anxiety characteristic of the disorder is also often accompanied by significant behavioural dysfunction. Such maladaptive behaviours are often seen during both social engagement and outside of social engagement. These behaviours are reflected, for example, in the use of

short-term coping mechanisms, such as avoidance or withdrawal, to minimise social engagement.

Stein and Stein (2008) reported SAD to be one of the most prevalent mental health disorders, particularly within Western culture, with rates highest amongst females and young adults. From the *National Survey of Mental Health and Wellbeing* conducted by the Australian Bureau of Statistics in 2007 (Australian Bureau of Statistics, 2008), SAD was reported to be the second most prevalent anxiety disorder, with over 100,000 Australians estimated to meet the diagnostic criteria of SAD within the previous 12 months. Outside of the 12-month scope, the survey determined that over 8% of Australians had at some point in their lifetime met SAD diagnostic criteria.

Social Anxiety Disorder is typically unremitting, with the disorder generally following a chronic course in clinical samples (Bruce et al., 2005). When engaging in treatment, around only 30% of SAD individuals experience remission of symptoms in a year, with up to 50% experiencing symptom remission within a few years of treatment (American Psychiatric Association, 2013). In consideration of the disorder's prevalence, the persistence of SAD symptoms in a large proportion of treatment engaging individuals, and the short term structure of the Australian 'Better access to mental health' scheme (providing financial rebate for only ten psychological therapy sessions per calendar year), it seems pertinent that the development of new or improved forms of SAD treatment is needed. New or improved treatments for SAD that demonstrate higher efficacy rates and achieve symptom remission within a shorter time frame is important for the management of this disorder.

The functional consequences of SAD are far-reaching, and include increased school drop-out, lower levels of employment and workplace productivity, and decreased socioeconomic status, well-being, and quality of life (Patel, Knapp, Henderson, & Baldwin, 2002). Individuals with the disorder are also more frequently single, unmarried or divorced,

and without children (Fehm, Pelissolo, Furmark, & Wittchen, 2005). In those that meet the diagnostic criteria, the disorder presents with sustained social withdrawal, avoidance and isolation. Significantly, while the impact of SAD is reflected in high levels of distress and poor social functioning, only half of SAD individuals in Western society seek treatment – and treatment engagement is typically sought after 15 to 20 years of symptoms being experienced (American Psychiatric Association, 2013). Further, most individuals with SAD do not seek treatment unless they develop a secondary diagnosis (Heimberg & Magee, 2014)

The early onset and chronic progression of SAD are particularly problematic as this creates vulnerability to the development of secondary disorders, including substance use disorders and major depressive disorder (Ruscio et al., 2008). The majority of those with SAD have additional diagnoses, with estimates of 70-80% of people with SAD meeting diagnostic criteria for additional mental health disorders (e.g. other anxiety disorders, MDD and substance use disorder; Magee, Eaton, Wittchen, McGonagle, & Kessler, 1996) with SAD often predating the comorbid diagnosis (Heimberg & Magee, 2014). The high level of comorbidity is particularly concerning as it has been shown to be associated with increased distress and poorer treatment outcomes (Teesson, Slade, & Mills, 2009). Suicidality is also of great concern in SAD, particularly for complicated SAD presentations in which the SAD diagnosis is accompanied by other mental health disorder diagnoses (e.g. MDD), with those individuals who experience comorbidities exhibiting higher rates of suicidality and attempts to suicide (Heimberg & Magee, 2014).

In the search for the most effective evidence-based treatments for SAD, research has investigated a number of treatment modalities and interventions, including various psychotherapeutic modalities (e.g. cognitive, interpersonal, dynamically oriented supportive psychotherapy), exposure work, social skills training, relaxation training, and a range of pharmacotherapies (Heimberg & Magee, 2014). Of these, there are two treatment modalities

that have demonstrated the highest efficacy in SAD treatment. These are the most commonly studied psychosocial intervention of cognitive behaviour therapy (CBT), in a form that combines exposure and cognitive restructuring (particularly the modality of cognitive-behavioural group therapy; CBGT), and the use of psychopharmaceuticals (Heimberg & Magee, 2014; Wong, Gordon, & Heimberg, 2012). However, while these two first-line treatments for SAD are well-established and demonstrate some efficacy for the treatment of the disorder, success rates are moderate at best. While these modalities demonstrate efficacy, there is an abundance of evidence for efficacy rather than demonstration of effectiveness in real world settings (Wong et al., 2012). It has been argued in the efficacy literature, that individuals with SAD who receive intervention or treatment in the research setting may not demonstrate the same outcomes as those who receive treatment in the non-research setting. Studies that are able to examine the generalisability of these research setting based efficacy rates, and the transportability of these empirically backed modalities to the real-world outpatient or private practice setting are required (Wong et al., 2012). In addition, it should be noted that for those not engaging in clinical treatment, greater than 60% follow a chronic course with the disorder for several years (American Psychiatric Association, 2013).

In sum, SAD presents as a chronic, persistent, poor treatment response rate disorder that is clinically distressing and can result in significant impairment and reduced quality of life. It is therefore not surprising that a large body of research has focused on gaining a better understanding of the disorder. To date, SAD has been studied at length in the laboratory setting and studies have targeted a range of topics including brain mechanisms, emotional subjective experience, physiology, biology, endocrinology, behavioural mechanisms, cognitive functioning to name a few. However, despite this extensive lab-based research, there is much to be understood about mechanisms of dysfunction in SAD that contribute to the onset, course and symptomology of the disorder. In particular, the lower ecological

validity of laboratory-based research limits insight into daily experiences of individuals with SAD, including how these underlying mechanisms present in daily life. Thus, while laboratory-based methodologies for exploring the disorder have contributed substantially to the understanding of SAD and have informed existing treatment strategies, other approaches are necessary in order to broaden our understanding of SAD and develop improved treatment modalities. One potentially valuable approach would be to assess SAD-related behaviour in the real world.

1.2. Studying Psychopathology in the 'Real' World

To fully understand the entire spectrum of human experience, research should aim to explore experiences as they naturally occur in the environment and understand how psychological processes and behaviours unfold across a number of settings (Barta, Tennen, & Litt, 2012). Until recently, the ability to collect ecologically valid data about people's daily behaviour in a real-world setting was limited. However, the development of real-world data capture methodologies, such as ecological momentary assessment (EMA), has allowed researchers to move away from predominantly focusing only on the aspects of human experiences that were accessible to laboratory experimentation and investigation (Mehl & Conner, 2012).

EMA methodology repeatedly samples participants' behaviour or experiences in the naturally occurring environment in real-time, most commonly these days, through the use of smartphone apps. EMA is therefore advantageous in that it involves real-world, dynamic data collection that is time-contingent and generalisable to real-life experiences. The frequent capture of data across time via EMA results in intensive longitudinal data. Such data provide insight into the dynamic nature of psychological processes and behaviours, observed in the way external (e.g. environmental) and internal (e.g. emotion regulation) forces alter the

course of the explored phenomenon (Mehl & Conner, 2012). In the context of SAD, this methodology offers an ideal way through which SAD can be explored outside of the laboratory environment, and potentially provides new insights in to understanding the daily life experiences of a disorder.

The introduction of the smartphone into society has enabled a number of advances in the field of EMA research. Indeed, the combination of smartphone apps and EMA design allowed advanced techniques to be used in the current thesis. Developments in the capabilities of modern smartphones have allowed EMA research to capture many forms of data including physiological data through external sensors, record live data via video recording, and location through GPS, and it can prompt participants through live signalling – all of which are welcome advances in EMA research. One such advance is the ability to install EMA research phone applications (e.g. SEMA3; Koval et al., 2019) onto participants' own smartphone devices, allowing for convenient, rapid, large-scale data collection at a reduced expense to a research project (Thai & Page-Gould, 2018).

The current research project in this thesis was designed to capitalise on the high level of smartphone ownership in the community, using a customised electronic application on participant's own smartphones, to collect real-world real-time dynamic data to contribute to the understanding of SAD outside of the laboratory environment. Employing EMA in SAD is particularly beneficial in assessing the experiences of the disorder as the symptom profile is often unpredictable, restricted to certain contexts or situations, and fluctuates over time (Walz, Nauta, & Aan Het Rot, 2014). This method allows reporting of specific aspects of the experience of the disorder (e.g. affect and behaviour) close in time to the naturally occurring environment in which SAD symptoms appear, thereby contributing to how we understand SAD across varying times and contexts (Kashdan & Farmer, 2014).

Despite the knowledge that EMA may make valuable contributions to the understanding of SAD and provides a novel way to explore the psychopathology and real-world experiences of individuals with the disorder, there have only been twelve EMA studies on SAD published to date. These existing studies have examined routine daily behaviours and emotions via portable computers, with questions assessing social activities and social contact, including frequency of particular social interactions (e.g. with romantic partners), ratings of social interactions in regard to positive and negative affect, exploration of anxiety and avoidance accompanying social interactions, symptom fluctuation, positive and negative feedback-seeking behaviours, the influence of affect on future health-promoting behaviours, associations between anxiety and fear and life goals and technological social interactions (e.g. phone and internet/chat) and face-to-face social engagement in SAD. Given the relatively small number of such studies however, it is apparent that there is currently still a limited understanding of the psychopathology of the disorder in real-life. This thesis therefore aimed to explore SAD beyond the laboratory using EMA techniques.

1.3. Conceptualisation of this Thesis

The overarching goal of this thesis, therefore, is to explore SAD in a unique way and address current gaps in the literature in order to provide a richer understanding of the social stress response in SAD and to inform clinical interventions. If the disorder is explored at length in the lab, and treatment effectiveness is not improving, then perhaps it is the lower ecological validity of lab based research and the lack of understanding of SAD in the real world that may be limiting new insights into the disorder and future development of effective treatments. The core aim of this thesis is to therefore explore the social stress response in SAD in the real world using EMA methodology. Specifically, with the understanding that social engagement can lead to considerable distress and functional impairment in daily life in

those with SAD, we aimed to explore how individuals with SAD respond to stress, specifically social stress, in daily life in order to contribute to our understanding of this highly prevalent and persistent disorder that is SAD.

The design of the research project from this thesis was complex, with data collection tapping into the social stress response in daily life through two major sources. The first was via EMA in the form of self-report surveys using a smartphone application for a one-week period to capture *daily life experiences* (i.e. subjective stress response). The second was via cortisol sampling which was undertaken at home on three days. This was designed to capture the diurnal profile of cortisol (i.e. physiological stress response) that is commonly understood for its presence in the body for arousing, activating, or adapting to stress.

A common pitfall of much of the daily life SAD research to date is the inability to ensure that all participants equally experience a significant social stressor or social engagement during their participation. It is difficult to capture how individuals with SAD respond to social stress using a traditional EMA design, as SAD is commonly associated with avoidance of such situations in daily life and the nature of the social experiences when encountered can be vastly different. Given the experience of SAD symptomology is often unpredictable and related to environmental stressors that experimenters cannot guarantee will occur organically during a study's data collection period, study designs may benefit from incorporating a social engagement during participation to ensure a social stressor is incurred and which is consistent for all participants.

To achieve this in the current thesis, we capitalised on the optimal experimental design of laboratory based research by having participants engage in a *lab-based acute social stress task* embedded in the middle of a week of EMA that makes for an 8-day experimental protocol. The laboratory visit involved a reliable and valid acute psychosocial stress task that all participants (healthy controls and those with SAD) completed. The inclusion of an acute

psychosocial stressor was done for two reasons: first, to ensure that all participants encountered a psychosocial stressor during participation that was also consistent for all participants (those with SAD compared to healthy controls), and second, to examine the broader profile of this acute social stress response in SAD in daily life, with a particular focus on the anticipation and recovery experiences related to the stressor. The choice of acute psychosocial stressor for the current study was the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993) which has been shown to be reliable, and is considered the gold-standard in scientific research for inducing a moderate acute stress response. Briefly, the TSST involves a task introduction where the participant is informed that they are to prepare for a 5-minute mock job interview in front of a panel of "managers". This is followed by an anticipation phase during which preparation for the interview occurs. There are also two active components that typically involve a 5-minute mock job interview followed by a 5-minute surprise arithmetic task.

This research project involves multilevel approach (e.g., between-person and within-person) of daily subjective and physiological data. The design captured 3 distinct phases: a *baseline phase* of subjective self-report and physiological (salivary cortisol) sampling, an *anticipatory phase* of subjective self-report and physiological (salivary cortisol) sampling which captured the individual's experience when they are made aware of an upcoming social task (i.e., TSST) to be completed the following day in the lab, and lastly a *recovery phase* of subjective self-report and physiological (salivary cortisol) sampling to capture the ongoing impact of an acute social stressor in the days following completion of the social task.

1.4. Outline of the Thesis

As noted, this thesis sets out to explore the acute social stress response in SAD and the subjective and physiological impact of social engagement outside of the constraints of a

single lab-based session. This research project is the first of its kind to explore SAD in a way that ensures a social stressor is encountered, so that the social stress response in SAD can then be explored in the daily life setting. While we implemented a lab-based acute social stress task to guarantee an acute social stress response occurred, the overarching goal of this project was to step outside of the research laboratory setting and examine SAD in the real-world. The acute stress response in SAD is largely examined in the laboratory environment, and this project aimed to contributing to a richer understanding of the subjective and physiological experience of SAD in response to social stress in the daily life setting. This thesis has implications for how we understand SAD, specifically how the subjective and physiological experience of the disorder transcribes across daily life in response to social stress.

This thesis begins with two literature review chapters providing the current research findings in relation to SAD (Chapter 2) and the research methodology of interest to this thesis, i.e., EMA (Chapter 3). More specifically, the first literature review chapter presents an up to date review of SAD including diagnostic criteria, characterising features, development, onset, course, risk and prognostic factors, gender differences, functional consequences, comorbidities, prevalence, current models of SAD, treatment and outcomes for SAD, and lastly a brief summary of existing neurobiological, cognitive and physiological stress response research for SAD. The purpose of this review is to outline current understanding of the disorder and provide a rationale for why expanding the currently dominant lab-based approach to understanding SAD to include other approaches is important. The second review chapter presents an introduction to EMA. This chapter highlights limitations of traditional research methods, provides an overview of EMA, including its strengths and weaknesses, its use in in clinical psychology, and lastly, reviews the existing EMA studies involving SAD.

This chapter provides a rationale for why the EMA methodology was utilised in the current research project.

A *general methodology* chapter (Chapter 4) is then presented that provides an overview of the entire research protocol, as well as general study information including ethics approval and recruitment. Participant information and clinical assessment details are also outlined including inclusion/exclusion criteria, screening procedures, diagnostic assessment and clinical assessment measures. A detailed section on the administration of the complete protocol is then provided. Finally, details of the statistical data packages used for data analysis are listed.

Immediately following the *general methodology* chapter in Chapter 5 of this thesis is the publication *An Introductory Guide to Conducting the Trier Social Stress Test* (Grace, Labuschagne, Rendell, Terrett, & Heinrichs, 2019) and accompanying supplementary material in the form of a laboratory manual that provides a detailed protocol for the administration the TSST. This publication and manual were not a core aim of this thesis. However, it became surprisingly apparent when seeking guidance for administration of the TSST that there was no clear and detailed methodology available for conducting the acute stress task, despite the methodology being cited thousands of times (Kudielka, Hellhammer, Kirschbaum, Harmon-Jones, & Winkielman, 2007) and the original protocol only containing a brief description (Kirschbaum et al., 1993). Experts in the field were consulted (including one of the original authors, Professor Clemens Kirschbaum) and many empirical papers and meta-analyses on the TSST were extensively reviewed. As a result of this process, an updated and detailed guide to assist researchers in conducting the TSST was established in the form of a step-by-step laboratory manual that was used for this thesis' research project. This peer-reviewed paper (see Chapter 5) also provides an overview of the potential impact of methodological variations on the stress (cortisol) response and is followed by the supplementary

material (the TSST manual). This introductory guide may be a useful and time-saving resource that may also improve the scientific standard and reliability of the reported psychobiological stress effects in future studies.

The next chapter (chapter 6) reports on *manipulation checks* the form of empirical data supporting the efficacy of the TSST as an acute social stressor in the current research project. Specifically, in this thesis, the TSST was used as a methodological tool, i.e., an acute psychosocial stressor, that was embedded with a larger EMA study design. Since the primary goal of this thesis focused on the EMA outcomes, this chapter provides evidence on the effectiveness of the TSST in eliciting an acute stress (cortisol) response in the participants. We directly compared the laboratory based subjective and physiological (salivary cortisol) acute stress response to the TSST of individuals with SAD to that of healthy controls, including information on the proportion of cortisol "responders" versus "non-responders" that is highly relevant for the main empirical analyses in the next chapter.

The *large-scale empirical paper* (Chapter 7) is then presented following a brief introduction of the study's rationale to remind the reader of the relevance and aim for the main empirical paper. This paper set out to reduce memory biases and increase the generalisability and ecological validity of the stress response data in SAD. The study aimed to contribute to the understanding of the acute social stress response in SAD in daily life, by examining associations between an acute social stressor and the subjective and physiological response in the broader context of daily life in SAD (i.e. the days leading up to and recovery from an acute social stressor). This chapter aimed to contribute a broadened understanding of the different experiences and ongoing effects of social stress in SAD in order to potentially inform new therapeutic approaches and decisions regarding management of the disorder.

The thesis then concludes with a final *discussion* chapter (Chapter 8) that reviews the thesis findings and broader limitations, as well as conclusions of the thesis, implications of this research project and potential future directions for research into SAD.

CHAPTER 2. A Review of Social Anxiety Disorder

2.1. DSM – 5 Diagnostic Criteria and Features Characterising Social Anxiety Disorder

Social anxiety disorder is categorised in the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5; American Psychiatric Association, 2013) as an Anxiety Disorder. The key marker of anxiety disorders is the shared feature of excessive fear and anxiety and the associated behavioural dysfunction. Fear pertains to the emotional response to imminent threat, real or perceived, and anxiety is the anticipation or worry toward future threat. While there is overlap between the two states, the presence of muscle tension, hypervigilance for future threat and avoidant or cautious behaviour is more commonly associated with anxiety, while fear is associated with more imminent danger and the activation of the bodies survival mechanisms, seen as surges of autonomic arousal that enable the fight flight mechanisms, and escape behaviours.

According to the DSM–5 (American Psychiatric Association, 2013), ten standard diagnostic criteria are cited in the classification of SAD, see Figure 2.1. Specifically, at the core of the disorder, is a marked fear or intense anxiety experienced by an individual when faced with a social situation or situations in which there is potential for judgement or scrutiny from others. These social situations can include commonplace interactions, such as meeting new and unfamiliar people or having a conversation with someone; being watched or observed (e.g. while eating or drinking) or during any performance task in front of others, such as giving a presentation or speech (American Psychiatric Association, 2013). The criteria further depict that the individual fears they will act in a manner or display symptoms of anxiety (such as blushing, sweating or staring) to a point in which they will be negatively evaluated (i.e. be humiliated or embarrassed or will likely offend or be rejected by others). Further to the fear of acting in a manner that will be negatively evaluated, those with SAD are also largely concerned with displaying attributes of themselves that they perceive as a deficit or flaw that will be critically evaluated by others. As such, a central fear of the

disorder is a strong belief that the individual possesses some deficit or flaw that if displayed will be subjected to judgement and scrutiny by others (Moscovitch, 2009).

The criteria emphasise that social situations typically always provoke fear or anxiety, though the degree of fear or anxiety experienced may vary across contexts. Some individuals may experience anticipatory anxiety around a social experience that they may continue over a number of weeks prior to social engagement. Often, SAD individuals may opt to avoid the fear or anxiety provoking social situations or opt to endure the situation – despite intense fear or anxiety. Avoidance behaviours in individuals with SAD can be overt, such as flat out refusal to attend social events, to a more subtle aversion of eye contact, redirection of attention onto others or over preparation for social events. When the risk of being negatively evaluated and the consequences of such negative evaluation are explored, the fear or anxiety experienced by the individual is typically judged as disproportionate to the actual risk or threat posed by the social situation and the sociocultural context, with the consequences of negative evaluation often largely overestimated by the individual. Inadequate assertiveness or an overly submissive demeanour may be seen in individuals with SAD, with possible demonstrations of rigidity in posture or avoidant eye contact. Individuals with SAD may be highly controlling of conversations or speak with low volume and increased softness, they may appear shy and less open to disclosure about themselves (American Psychiatric Association, 2013). The use of substances, such as alcohol consumption before a social engagement, is common in SAD. Lastly, disorder criteria highlight that the fear or anxiety symptoms last typically longer than 6 months and are enduring, with the effects of this causing clinically significant distress and impairment in social, occupational or other important areas of functioning. The fear or anxiety is not attributable to substance use or another medical condition, nor is it better explained by the symptoms of another medical condition; alternatively, if another medical condition is present, the fear or anxiety is clearly

unrelated or excessive (American Psychiatric Association, 2013). It is also specified whether the social anxiety is performance only, or restricted to speaking or performing in public – in such cases, individuals with performance SAD do not avoid or fear social situations without a performance element to them (American Psychiatric Association, 2013).

In addition to the existing diagnostic criteria for SAD, revised models of the disorder (e.g. see Heimberg, Brozovich, & Rapee, 2014) stipulate the experience of social anxiety disorder is not *all* about the negative. There is an increasing body of empirical support for the disorder, in part, being a response to the fear of positive evaluation, in addition to the well documented fear of negative evaluation. While fear of negative evaluation embodies apprehension and distress felt around potential negative evaluation by others (Weeks, Heimberg, Rodebaugh, Goldin, & Gross, 2012) fear of positive evaluation is its own distinct construct, albeit related. Heimberg et al (2014) propose the fear of positive evaluation as an additional cognitive component in the maintenance of the disorder, with the fear of positive evaluation pertaining to a felt sense of apprehension or dread around being looked upon favourably and publicly, leading to feelings of being in the spotlight and compared to others (Weeks et al., 2012).

Diagnostic Criteria for Social Anxiety Disorder - 300.23 (F40.10)

a. Marked fear or anxiety about one or more social situations in which the individual is exposed to possible scrutiny by others. Examples include social interactions (e.g., having a conversation, meeting unfamiliar people), being observed (e.g., eating or drinking), and performing in front of others (e.g., giving a speech).

   Note: *In children, the anxiety must occur in peer settings and not just during interactions with adults.*

b. The individual fears that he or she will act in a way or show anxiety symptoms that will be negatively evaluated (i.e., will be humiliating or embarrassing; will lead to rejection or offend others).

c. The social situations almost always provoke fear or anxiety.

   Note: *In children, the fear or anxiety may be expressed by crying, tantrums, freezing, clinging, shrinking, or failing to speak in social situations.*

d. The social situations are avoided or endured with intense fear or anxiety.

e. The fear or anxiety is out of proportion to the actual threat posed by the social situation and to the sociocultural context.

f. The fear, anxiety, or avoidance is persistent, typically lasting for 6 months or more.

g. The fear, anxiety, or avoidance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

h. The fear, anxiety, or avoidance is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition.

i. The fear, anxiety, or avoidance is not better explained by the symptoms of another mental disorder, such as panic disorder, body dysmorphic disorder, or autism spectrum disorder.

j. If another medical condition (e.g., Parkinson's disease, obesity, disfigurement from burns or injury) is present, the fear, anxiety, or avoidance is clearly unrelated or is excessive.

Specify if: **Performance only:** If the fear is restricted to speaking or performing in public.

Figure 2.1. DSM–5 diagnostic criteria for SAD (Social phobia), r*eprinted with permission from the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, (Copyright 2013). American Psychiatric Association.*

2.2. Development, Onset and Course

The onset of SAD may occur following an experience of humiliation or significant stress, such as bullying or throwing up during a public performance or speech (Rapee & Spence, 2004) or the disorder may develop insidiously across time. In studies across the United States and Europe, the SAD has been shown to also arise from a childhood history of shyness or social inhibition (Essex, Klein, Slattery, Goldsmith, & Kalin, 2009). Age of onset for the disorder is predominantly in early childhood and adolescence (Fehm et al., 2005; Schneier, Johnson, Hornig, Liebowitz, & Weissman, 1992) with SAD typically unremitting, following a chronic course in clinical samples (Bruce et al., 2005). The first onset of the disorder occurring in adulthood is rare (Grant et al., 2005) with cases emerging in adulthood likely arising following a significantly stressful or humiliating event or the adoption of new social roles (e.g. an occupational promotion). Among those individuals with SAD who have sought clinical care, the disorder presents as chronic and persistent across time. Young adults exhibit increased levels of social anxiety toward specific situations (Gretarsdottir, Woodruff-Borden, Meeks, & Depp, 2004), whereby in comparison older adults show decreased social anxiety however this occurs across a broader range of situations. Within older adult populations, the occurrence of social anxiety may arise from concerns around declines in sensory functioning (e.g. loss of hearing), appearance (e.g. visible physical conditions such as tremors), decreased mobility, declining cognitive performance (e.g. forgetfulness), medical conditions or incontinence (American Psychiatric Association, 2013).

2.3. Prevalence

The maintenance of informed and current estimates of the prevalence, comorbidity and treatment-seeking behaviors associated with SAD is essential for appropriate clinical practice and research. The *National Survey of Mental Health and Wellbeing* was conducted

by the Australian Bureau of Statistics in 2007 (Australian Bureau of Statistics, 2008). The survey collected a plethora of mental health and wellbeing information from a nationally representative random sample of 8841 Australians aged between 16 to 85 years of age. The survey assessed the prevalence of the diagnostic criteria for SAD and related mental health disorders over both 12 month and lifetime periods using the World Mental Health Composite International Diagnostic Interview (Crome et al., 2015). The prevalence of mental disorders describes the proportion of people in any given population who have met the diagnosis criteria for a mental disorder at a point in time.

According to the survey, 3.2 million Australians (20%) had a 12-month mental disorder, meaning they had met diagnostic criteria for a lifetime mental disorder and had experienced symptoms within the previous 12 months. Of these, 2.3 million (14.4%) of Australians between 16 to 85 years of age met criteria for an anxiety disorder within the previous 12 months. Of the anxiety disorders, SAD accounted for 4.2% of Australians with a 12-month mental disorder, the second highest 12-month prevalence behind post traumatic stress disorder (PTSD; see footnote for PTSD and obsessive compulsive disorder [OCD] diagnostic classification)[1] at 6.4%; see Figure 2.2. Outside the 12-month scope, SAD criteria were met by 8.4% of Australians at some stage in their lifetime. A clear majority (70%) of individuals meeting the SAD diagnostic criteria within the previous 12 months also experienced a comorbid mental health disorder within their lifetime. Primarily, comorbidity was associated with disorders such as Major Depressive Disorder (MDD), generalised anxiety disorder (GAD) and PTSD. Significant comorbidity between SAD and substance abuse was also observed. Of those reporting SAD as their primary burden, a mere 20% sought treatment for the disorder (Crome et al., 2015). Women experienced 12–month mental

[1] The author notes that PTSD and OCD are no longer classified as anxiety disorders in the most recently published DSM – 5 (American Psychiatric Association, 2013)

disorders at a higher rate than men (22% and 18% respectively) and higher rates of anxiety disorders (18% and 11% respectively). Specifically, women experienced SAD at higher rates compared to men (5.7% compared with 3.8%). Across age groups, the prevalence of 12-month mental disorders varies largely, with those in younger ages groups experiencing higher rates of mental disorder. Anxiety disorders exhibited the highest prevalence across all age groups, with the highest prevalence of anxiety disorders appearing in the 35 to 44 years age bracket (Australian Bureau of Statistics, 2008).

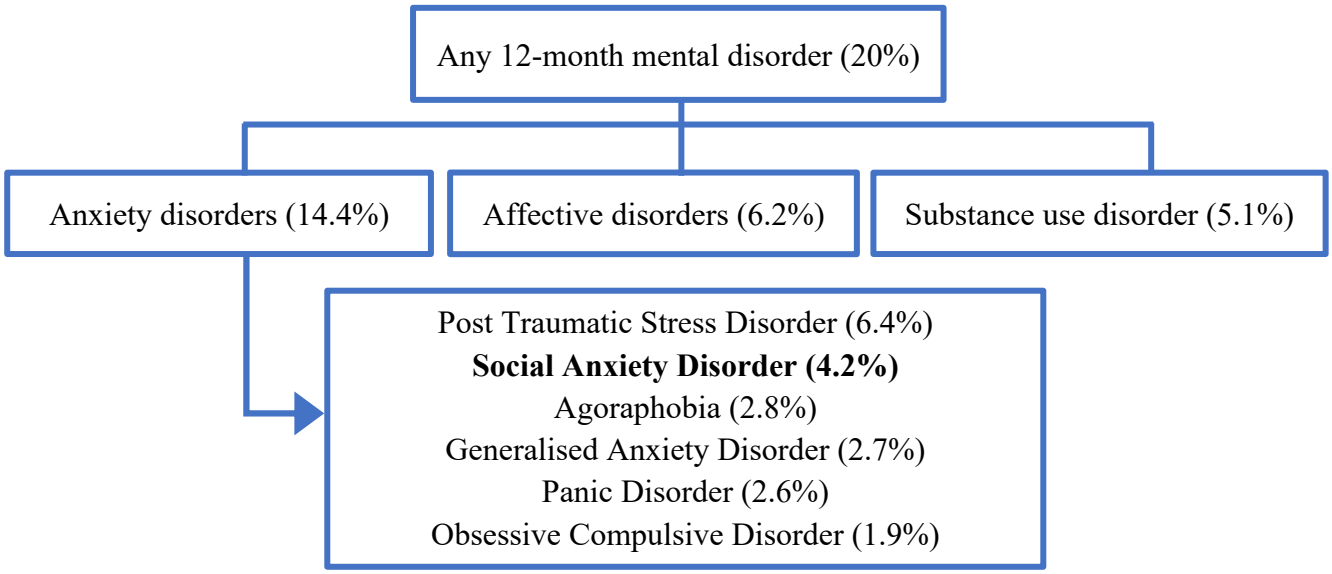


Figure 2.2. Depiction of 12-month prevalence of mental disorders from the National Survey of Mental Health and Wellbeing in Australia

2.4. Functional Consequences and Gender Related Influences in Social Anxiety Disorder

There are significant functional consequences associated with SAD, including increased rates of school drop-out, lower employment levels, decreased workplace productivity and reduced socioeconomic status (Patel et al., 2002). Further, SAD individuals report decreased well-being and lower quality of life (Patel et al., 2002). Individuals with the disorder also experience impairment in intimate relationships, with SAD associated with increased likelihood for being single, unmarried or divorced and not having children (Fehm

et al., 2005). In the aging population, SAD may impede the uptake of and participation in leisure activities and result in increased isolation. The employment status of an individual with SAD is also a significant predictor for the persistence of the disorder, i.e. level of exposure to the workplace setting may potentially increase comfort on the job and engagement in the social setting (American Psychiatric Association, 2013)

The functional consequences and experience of SAD also largely varies between gender. Differences in gender for SAD exist in the prevalence, severity and presentation of the disorder. Regarding prevalence, females are consistently reported as having increased rates of SAD compared to males, with estimates of a 3:2 ratio ((Brook & Schmidt, 2008; Rapee & Spence, 2004). Research has cited gender roles, particularly the increased association between anxiety symptoms with feminine traits (compared to masculine traits) as a potential explanation for the disparity between genders and the higher rates of SAD in females (Brook & Schmidt, 2008; Muris, Meesters, & Knoops, 2005). Gender has also been reported as having a moderating role on family adversity and risk for developing SAD, with an increased risk for development of SAD strongly associated with family adversity according to gender (Dewit et al., 2005). Specifically, males were twice as likely to develop SAD than females if there was the absence of a close, supportive, confidant relationship in childhood. Females were up to two times more likely than males to develop the disorder if they had been exposed to marital conflict while growing up, and further, if their mother had suffered from mental illness they were twice as likely to experience SAD (Dewit et al., 2005). Females also demonstrated an increased probability for the disorder if they had reported physical abuse from their father (Dewit et al., 2005). Evidence supports significant gender differences in the experience and response to negative child-parent interactions play a role in the difference prevalence rates of SAD between males and females.

Regarding severity, treatment seeking females with SAD report increased clinical severity for SAD symptoms when compared to men (Asher, Asnaani, & Aderka, 2017; Turk et al., 1998). Females self-report higher levels of anxiety experienced in anticipation for and during social encounters, and higher levels of social fear when compared to males (Crome, Baillie, & Taylor, 2012). Importantly of note, females with SAD report a significantly higher endorsement to die and increased suicidality when compared to males. This finding is consistent even when controlling for comorbid depression, and is attributable to the experience of SAD regardless of the presence of comorbid depression (Asher et al., 2017).

In the employment setting, females are reported to have lower levels of workforce employment, less likelihood for full time employment, and lower personal income when compared to males (Asher et al., 2017). Gender difference in the types of fear endorsed by males and females indicate women have increased fear around interactions involving authority figures, work presentations in an office setting, working while being observed, entering a room with others already seated and reporting to a group. These fears are common to employment and workplace experiences and settings, and as such may be indicative as to why gender difference exist in employment and income (Turk et al., 1998). In the relationship and interpersonal domain, males with SAD are reported to typically have an increased likelihood of fearing dating and are more likely to report living along and being single (Turk et al., 1998). However, among females with SAD but not males, diminished personal disclosure and openness in romantic partnerships and close friendships has been reported (Cuming & Rapee, 2010). Males are more likely to have oppositional defiant disorder or conduct disorder, or to use illicit drugs or alcohol in order to relieve symptoms of the disorder, while females with SAD are more likely to experience comorbid depression, bipolar disorder and other anxiety disorders (Ruscio et al., 2008; Turk et al., 1998).

2.5. Comorbidity

Around 70-80% on individuals with SAD meet the criteria for additional diagnoses, with SAD suggested to predate the additional comorbid mental disorder diagnoses on most occasions (Heimberg & Magee, 2014). Specifically, the comorbidity of SAD with other anxiety disorders, MDD and substance use disorders is high, with the onset of SAD said to typically occur ahead of other disorders, except in cases of specific phobia and separation anxiety disorder (Beesdo et al., 2007). It is possible the sustained social withdrawal, avoidance and isolation that arises in the course of social anxiety may lead to the development of MDD. Particularly for the older adult population, comorbidity between SAD and depression is high (King-Kallimanis, Gum, & Kohn, 2009). Contrary to the existing findings from previous reviews examining depressive disorders and SAD, a recent Jacobson and Newman (2017) systematic review and meta-analysis examining the prospective relationship between anxiety and depression found depressive disorders may be prodromes for SAD. Jacobson and Newman (2017) concluded that depressive disorders predicted SAD more strongly that the opposing and commonly held belief SAD predicted MDD.

Frequently in SAD, substances are used as a form of self-medication for the fear experienced around social engagements, however the repercussions of substance use such as intoxication or withdrawal (e.g. trembling) have potential to act as a further source of social anxiety. In addition to using substances to cope with the negative affect experienced around social engagement, research suggests other motives may exist in the problematic use of substances in SAD, including for social enhancement, to increase positive affect and social conformity (Buckner, Eggleston & Schmidt, 2006; Lewis et al., 2008)

High comorbidity also exists between SAD and bipolar or body dysmorphic disorder. The presence of SAD is also common in children with high functioning autism (Steensel, Bögels, & Perrin, 2011) and selective mutism. Significant concern arises from the high levels

of comorbidity with SAD as comorbidity is typically a signifier for increased distress and poorer treatment outcomes (Teesson et al., 2009). Compared to individuals with uncomplicated SAD (no secondary diagnoses), SAD individuals with comorbid disorders exhibit higher rates of suicidality and suicide attempts, report greater impairment and have an increased likelihood of using medication to control the symptoms of the disorder (Heimberg & Magee, 2014). The links between social anxiety and negative interpersonal relationships and employment outcomes appeared relevant only if social anxiety was experienced within the previous year. This implies the possibility that once an individual's social anxiety has been resolved the associated deficits may also recede.

2.6. Preeminent Models of Social Anxiety Disorder

Several prominent psychological models of SAD have been developed that identify specific factors attributed to the aetiology and maintenance of SAD. Several cognitive and cognitive behavioural maintenance models of the disorder developed across the mid 1990's and 2000's specifically identify cognitive and behavioural factors theorised to contribute to the *maintenance* of SAD. The most prominent traditional *maintenance* models include:

i. A cognitive model of social anxiety disorder (Clark & Wells, 1995);
ii. A cognitive behavioral model of social anxiety disorder: Update and extension (Heimberg, Brozovich, & Rapee, 2014);
iii. Cognitive factors that maintain social anxiety disorder: A comprehensive model and its treatment implications (Hofmann, 2007); and
iv. A cognitive-behavioural model of anxiety in social phobia (Rapee & Heimberg, 1997)

These models generated an expanse of research and subsequent treatment protocols with demonstrated efficacy (see Rapee, Gaston, & Abbott, 2009), informing the understanding of the specific maintenance factors of SAD. The more recent (Clark & Wells, 1995; Hofmann, 2007; Rapee & Heimberg, 1997) and revised (Heimberg et al., 2014) cognitive behavioural maintenance models propose SAD individuals engage with maladaptive and dysfunctional cognitive (e.g. post-event processing) and behavioural (e.g. avoidance) processes that contribute to maintaining a fear or anxiety response when social situations are encountered (Wong & Rapee, 2016). The existing models are relatively consistent in the factors they propose to maintain SAD, with the key maladaptive cognitive and behavioural processes that maintain the disorder occurring according to proximity to the social event (i.e. before, during or after social engagement). In sum, the key SAD maintenance processes of the disorder include, (i) cognitive processes, such as anticipatory processing, negative social-evaluative cognitions, cognitive avoidance, attentional bias to threat (i.e. self-focus and external threat) during social engagement, and post-event processing after social events, and (ii) behavioural factors, such as anxiety related performance deficits, safety behaviours, performance deficits from a lack of social skills, escape behaviours, avoidance behaviour before social engagements (Wong & Rapee, 2016).

A limitation of maintenance models of SAD is that the models do not specifically address the aetiological basis of SAD or how the disorder and its maintaining factors are developed. Separate to the maintenance models of SAD, a collection of *aetiological* models of SAD have also been developed, such as:

i. The aetiology of social phobia: empirical evidence and an initial model (Rapee & Spence, 2004); and
ii. Social phobia (social anxiety disorder) (Hofmann & Barlow, 2002).

Aetiological models of SAD identify the risk factors, such as biological, psychological and social components, that increase the likelihood for the development of SAD rather than the factors that contribute to the maintenance of the disorder. Prior to 2016, no model of SAD existed in which there had been an attempt to comprehensively integrate the key aetiological and maintaining factors of SAD in the current literature. Wong and Rapee (2016) proposed a united model of these aetiological and maintenance factors in a systematic review of the current literature that led the new integrated aetiological and maintenance (IAM) model of SAD (see Figure 2.3).

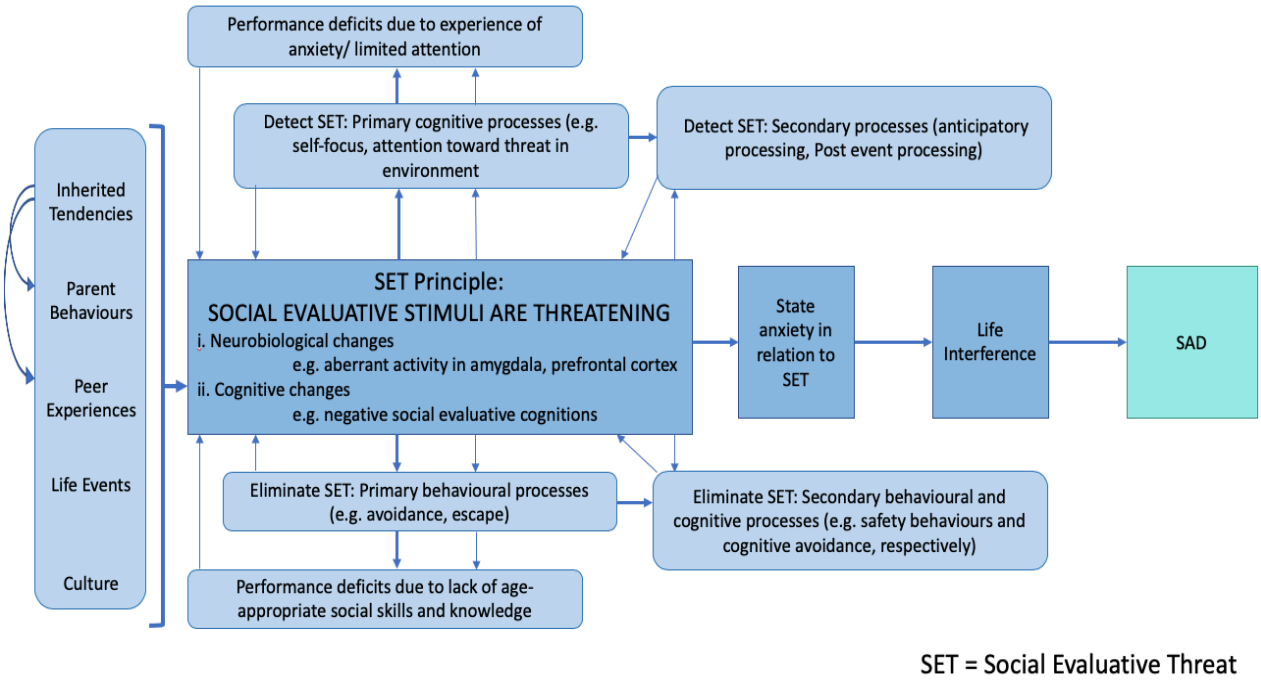


Figure 2.3. The integrated aetiological and maintenance (IAM) model for SAD (figure adapted from original, see Wong & Rapee, 2016) Note. Bold arrows indicate aetiological pathways, all other arrows represent key maintaining pathways.

From the review, the key factors identified to be involved in the aetiology of SAD included a widespread acceptance for the importance of temperament (e.g., behavioural

inhibition, shyness, negative affectivity) and genetic predispositions (e.g., specific vs. broad vulnerability to psychopathology) as early risk predictors for SAD. The literature defined early cognitive biases (e.g., cognitive bias, distortions and increased in self-focussed attention), the experience of negative life or social events (e.g., loss and grief, interpersonal conflict), relationships between child and primary caregiver, and relationships with peers as important influences in the development of SAD (Wong & Rapee, 2016). Less influence was attributed to additional biological factors, general learning and performance deficits, and cultural factors in the development of SAD.

The IAM model of SAD proposes a model that identifies specific aetiological factors that most likely exert their influence on the development of SAD in early life, and the aetiological factors determine the level of threat individuals assign to social-evaluative stimuli with this threat value assigned to social-evaluative stimuli thereby guiding an individual in their environment (e.g. the social-evaluative threat (SET) principle). The model proposes specific aetiological factors (e.g. inherited tendencies, peer experiences, parent behaviour, life events and culture) contribute to increases in the threat value, and subsequently results in the development of maladaptive cognitive process such as self-focus and attention toward external threat in the environment to identify potential threat in the environment (Wong & Rapee, 2016) along with maladaptive behavioural processes to eliminate the social evaluative threat (e.g. avoidance and escape behaviours).

The model suggests the development of secondary maladaptive cognitive and behavioural processes in SAD as a result of the primary cognitive and behavioural processes described. These secondary processes continue to maintain high threat values assigned to social evaluative stimuli, accommodates the development of performance deficits due to lack of social skills, increased anxiety and reduced attention, and leads to the development of further maladaptive cognitive processes which facilitate the continued detection of social-

evaluative threat (e.g. anticipatory or post-event processing) and the use of cognitive (e.g. cognitive avoidance) or behavioural processes (e.g. the use of safety behaviours) that aim to eliminate potential social-evaluative threat (Wong & Rapee, 2016).

The model links the developmental aetiological pathways to primary cognitive and behavioural processes, performance deficits and secondary maladaptive processes in order to provide novel insights into the connection between aetiological factors of SAD and other factors linked to the maintenance of SAD. The model serves as a new account of potential risk factors for SAD, and how these serve to increase the development of the key maintenance factors of the disorder along with increasing the likelihood of onset of SAD. The novel IAM model highlights that both the aetiological and maintaining factors of SAD contribute to the development, onset and course of the disorder (Wong & Rapee, 2016). Consequently, the IAM model proposes the integration of both aetiological and maintenance theory of SAD is essential for understanding the disorder and informing the development of comprehensive and effective treatments. The IAM model provides a novel and integrative conceptualisation of SAD and the factors to consider in the design of the empirical studies of this thesis. For example, the main empirical study of this thesis (Study 2, Chapter 7) accounted for some aetiological factors including inherited tendencies (i.e., personality measure) and life events (i.e., childhood trauma questionnaire), and assessed daily experiences relating to threat sensitivity in the environment, negative social evaluative cognitions, anticipatory experiences, and anxiety. Although the scope of this thesis was not to test the model itself, it nevertheless provided invaluable insights for the design of this thesis.

2.7. Risk and Prognostic Factors

A number of temperamental, genetic, physiological and environmental factors are cited as risk and prognostic factors for SAD. Estimates from several studies determine genetic factors may account for 30-60% of the variance in SAD (Andrews et al., 2003;

Craske et al., 2017). Specifically, genome scans have demonstrated specific loci may predispose individuals to social anxiety, along with demonstrating shared genetic risk associations with other dispositional traits (e.g. between social anxiety and neuroticism and extraversion; Stein et al., 2017). While the above estimates report genetic factors account for a large proportion of variance in SAD, it is suggested only a small component of the observed variance is actually attributed to social fear risk factors, with variance more likely reflecting more common genetic risk factors, such as those associated with anxiety, depression or other dispositional traits, like personality (Rapee & Spence, 2004). Additional traits suggested to predispose individuals to SAD are behavioural inhibition and fear of negative evaluation (American Psychiatric Association, 2013). Trait anxiety and harm avoidance are also associated with higher of SAD (Bienvenu, Hettema, Neale, Prescott, & Kendler, 2007). There is also a confirmed heritable basis to SAD (Stein et al., 2017), with first degree relatives having two to six times greater likelihood of SAD.

While there is evidence for a strong genetic influence on the traits that predispose an individual to SAD (Fox et al., 2005), this influence is subject to gene-environment interaction. Individual liability for the disorder involves the interaction of both non-specific genetic factors (e.g. neuroticism), disorder specific genetic factors (e.g. fear of negative evaluation; Bögels & Stein, 2009) and environmental risk factors. Environmental risk factors cited as playing a relevant role in SAD include: the experience of adverse life events, parental, familial, cultural and social environments, and gender roles (Brook & Schmidt, 2008). Specifically, parental traits that include overcontrol, rejection, the absence of warmth and overprotection, along with anxious attachment styles, and anxiety disorders in general are shown to be environmental risk factors for SAD and have associations to the disorder (Brook & Schmidt, 2008; Elizabeth et al., 2006; McLeod, Weisz, & Wood, 2007). Children who exhibit increased behavioural inhibition are also more likely to be susceptible to

environmental influences, including socially anxious modelling in their parents (Aktar, Majdandžić, de Vente, & Bögels, 2013). Further, parental psychopathology has been shown to influence a child's social and emotional development. Research indicates parents with SAD are significantly more likely to have children with SAD than can be attributed to genetic factors alone (Brook & Schmidt, 2008). Tiet et al (2001) determined childhood psychosocial adversity or maltreatment had no causative role in the development of SAD, however these are included as risk factors for the disorder. Despite the lack of evidence for a causative role, losses (e.g. death or separation), negative family environment, family violence, abuse history (physical and sexual), illnesses in childhood and bullying are all thought to contribute to the environmental etiology of SAD (Brook & Schmidt, 2008), with these varying traumatic advents and childhood adversities potentially apart of the conditioning response for SAD.

2.8. Treatment and Outcomes for Social Anxiety Disorder

Despite the significant distress and impairment in social functioning associated with this persistent disorder, treatment seeking is only observed in around half of the individuals with SAD in Western societies, with treatment typically also not sought until up to 15-20 years of experiencing social anxiety symptoms (Heimberg & Magee, 2014). A broad range of treatment interventions for SAD have been explored, including but not limited to, social skills training, cognitive therapy, relaxation training, exposure, interpersonal psychotherapy, dynamically oriented supportive psychotherapy, and various pharmacotherapies (Heimberg & Magee, 2014). Cognitive behaviour therapy (CBT) and the use of psychopharmaceuticals have both demonstrated the highest efficacy in the treatment of SAD, with both well established as the primary treatments for the disorder (Boyce et al., 2015; Hofmann & Otto, 2017; Ipser, Kariuki, & Stein, 2008). A brief overview of the two treatments will follow.

2.8.1. Cognitive behavioural therapy. Psychotherapeutic intervention for SAD using CBT is primarily through a combination of psychoeducation (understanding the disorder and the experience of SAD), cognitive restructuring, and exposure. The therapeutic modality theorises that the way one thinks (e.g. cognition) and the way one acts (e.g. behaviour) affects the way one feels (e.g. affect) - known as the cognitive triangle. The key tenet of the CBT model is that emotional reactions to a situation are caused by the interpretation of that situation, and not the situation itself. CBT aims to help individuals understand their thoughts (particularly *negative automatic thoughts*), encourage flexible or balanced thinking and perspective taking, and foster behavioural change.

Cognitive restructuring is part of the psychotherapeutic process in which the individual learns to identify and dispute irrational, distorted or maladaptive thoughts or beliefs, commonly known as unhelpful thinking styles, cognitive distortions or thinking traps. A plethora of techniques for cognitive restructuring exist, with some of the more commonly recognised techniques including Socratic questioning (e.g. Is this thought realistic? Am I basing my thoughts on facts or on feelings?) and thought diaries (exploring the activating event (A), Beliefs/thoughts (B), and Consequences/feelings (C) in order to work towards a more balanced interpretation of the activating event).

Exposure work involves graded exposure to feared social situations, in order to gradually decrease anxiety, condition the individual to the feared environment, decrease anxiety related expectancies and increase confidence and coping skills for managing anxiety provoking social situations. Together with cognitive restructuring to address underlying maladaptive thinking that arises through exposure, the technique is a well-researched and supported treatment for SAD. Several exposure strategies exist, dependent upon the individual circumstances and anxiety level. In-vivo exposure, guided imagery, role playing, virtual reality technology, flooding (non-graded exposure; immersion into the most feared

scenario) or systematic desensitisation are all common exposure techniques. Sessions typically involve exposure pre-processing (e.g. negotiating details of exposure; eliciting automatic thoughts) followed by the exposure activity and concluding with exposure post-processing (e.g. review of goal attainment, review of automatic thoughts elicited, and subjective units of distress scale assessment)(for a current step-by-step SAD treatment guide, see Heimberg & Magee, 2014).

2.8.2. Psychopharmaceuticals. Many RCTs have demonstrated efficacy for medications for SAD (Bandelow, Michaelis, & Wedekind, 2017). Pharmacotherapy for SAD has been refined to six drug classes with demonstrated efficacy for the treatment of the disorder, including the most commonly used serotonin selective reuptake inhibitors (SSRIs), along with serotonin and norepinephrine reuptake inhibitors (SNRIs), irreversible monoamine oxidase inhibitor (MAOIs), reversible inhibitor of mono-amine oxidase A (RIMA), calcium modulators, and benzodiazepines. SSRIs work to block the reuptake transporters at the presynaptic level, increasing synaptic serotonin and consequently serotonin transmission. SSRIs have demonstrated short-term and long term treatment efficacy across a broad spectrum of anxiety disorders, and of the drug classes are typically well tolerated; for these two reasons, SSRIs are considered the first-line medication approach for SAD (Baldwin et al., 2014; Pilling et al., 2013).

Serotonin and norepinephrine reuptake inhibitors (SNRIs) have demonstrated some efficacy for the treatment of SAD. SNRIs work to inhibit the reuptake of serotonin and norepinephrine back into the cells that released them, in order to maintain the levels of these two chemicals in the brain. SSRIs and SNRIs have similar pharmacological properties, tolerance profiles and reported efficacy and as such share the first-line drug class for treatment of SAD (Blanco, Bragdon, Schneier, & Liebowitz, 2013). However, there is some

evidence that the SNRI venlafaxine used to treat SAD is less tolerated than the SSRIs that have demonstrated efficacy in SAD (Baldwin et al., 2014).

The calcium modulator Pregabalin has also demonstrated efficacy in both acute treatment and prevention of relapse in SAD (Bandelow et al., 2017). Phenelzine, a traditional irreversible monoamine oxidase inhibitor (MAOI) has demonstrated efficacy in SAD, however the notable side effects and following of dietary restrictions has limited its use. Phenelzine overdose is potentially fatal, and potential interactions between MAOIS and other drug classes such as SSRIs can be harmful. As such, Phenelzine is typically reserved for individuals who have not responded to or tolerated other treatment approaches (Baldwin et al., 2014). The reversible inhibitor of mono-amine oxidase A (RIMA), Moclobemide has also demonstrated efficacy in the treatment of SAD, with the reversibility of the drugs action resulting in the added benefit of reducing the need for dietary restrictions seen in MAOIs when used at a lower dose. The drug class of benzodiazepines have demonstrated efficacy for the treatment of SAD (Blanco et al., 2013). Benzodiazepines enhance the action of the neurotransmitter, Gamma Amino Butyric Acid (GABA). Benzodiazepines are, however, notorious for potentially harmful sedation and cognitive impairment in the short and long term, along with the possibility for tolerance and drug dependence to occur particularly in predisposed individuals. Typically, the use of benzodiazepines is only in the cases of individuals who have been non-responsive to a minimum of three prior treatments (e.g. SSRI, SNRI and psychotherapy). Benzodiazepines are recognised as potentially effective, but generally reserved for short term use (Baldwin et al., 2014). The pharmacological recommendations for the treatment of SAD, are shown in table 2.1. This includes drug class, drug name, demonstrated efficacy in RCTs, recommended dosage and adverse effects.

Table 2.1

Current Pharmacological Treatments for SAD

Drug Class	Drug	Efficacy in RCTs	Recommended Dosage	Adverse Effects
Selective serotonin reuptake inhibitors; SSRI	Citalopram	✓	20 – 40mg	Some reported accounts of jitteriness, nausea, restlessness, headache, fatigue, appetite changes (+ and -), weight changes (+ and -), tremor, sweating, QT_c prolongation (QT interval relates to a specific element of electrocardiogram (ECG), the effect is important as it associated with potential concern in cardia functioning), sexual dysfunction, diarrhea, constipation, and other side effects
	Escitalopram	✓	10 – 20mg	
	Fluvoxamine	✓	25 – 50mg	
	Paroxetine	✓	20 – 50mg	
	Sertraline	✓	50 – 150mg	
Selective serotonin norepinephrine reuptake inhibitors; SNRIs	Venlafaxine	✓	75 – 225mg	Reported accounts of jitters, nausea, restlessness, headache, fatigue, appetite changes (+ and -), weight changes (+ and -), tremor, sweating, sexual dysfunction, diarrhea/constipation and urinary problems.
Calcium modulator	Pregabalin	✓	150 – 600mg .	Reported accounts of dizziness, somnolence, dry mouth, edema, blurred vision, weight gain, constipation, euphoria, increased appetite, difficulty with concentration/ attention, withdrawal symptoms if abruptly discontinued, other side effects.
Irreversible monoamine oxidase inhibitor; MAOI	Phenelzine	✓	15 – 90mg	Evidence for dry mouth, nausea, diarrhea or constipation, headache, drowsiness, insomnia, dizziness or light-headedness, low blood pressure, reduced sexual desire or difficulty reaching orgasm, weight gain. Dietary restrictions must be observed. Overdose is potentially fatal. Interactions involving traditional MAOIs and SSRIs and clomipramine can be harmful.
Reversible monoamine oxidase A inhibitor; RIMA	Moclobemide	✓	300 – 600mg	Reported accounts of restlessness, insomnia, dry mouth, headache, dizziness, gastrointestinal symptoms, nausea and other side effects.
Benzodiazepine	Clonazepam	✓	0.5 – 6mg	Sedation, cognitive impairment, potential for abuse.
	Bromazepam	✓	3 – 27mg	Sedation, cognitive impairment, potential for abuse.

2.8.3. Summary of treatment outcomes. Despite primary treatments for SAD being well-established and demonstrating treatment efficacy, the evidenced-based treatments of choice leave significant room for improvement and largely demonstrate efficacy but not necessarily effectiveness (Wong et al., 2012). Many individuals seeking treatment for SAD do not do so until late adulthood, with community estimates reporting approximately on 30% of SAD individuals engaging with treatment experience remission of symptoms within 12-months, while approximately 50% experience symptom remission within a few years. Further, those who seek treatment for SAD largely only do so after the onset of a secondary disorder (e.g. MDD). For SAD individuals who are non-treatment seeking or do not receive specific treatment, around 60% of these individuals will continue on a chronic course and remission can take up to several years or more (American Psychiatric Association, 2013). The experience of SAD is clinically distressing and can result in significant impairment and reduced quality of life. With novel insights such as the IAM model of SAD, and a continued movement toward further understanding of the disorder it is the intention of this thesis to stimulate new research to improve treatment strategies for SAD.

2.9. Summary of Neurobiological, Cognitive and Physiological Stress Responses in SAD and the Primary Focus of this Thesis

Neurobiologically, SAD is characterised by widespread generally heightened brain reactivity when compared to healthy controls. With the primary focus of this thesis the subjective psychological and peripheral salivary cortisol stress response in SAD, a full review of central nervous system neurobiological (i.e. brain) function and structure in SAD was beyond the scope of this thesis. We briefly summarise the findings from two key reviews of neuroimaging studies in SAD (Brühl, Delsignore, Komossa, & Weidt, 2014; Etkin & Wager, 2007) to highlight the wide range of human brain functions impacted by the experience of

SAD. Etkin and Wager's (2007) review of functional neuroimaging studies demonstrated that overactivity in the brain in SAD compared to healthy controls was primarily observed in the 'fear circuit' (e.g. amygdala, insula, anterior cingulate and prefrontal cortex, see Marek, Strobel, Bredy, & Sah, 2013 for a fear circuit review). With neuroimaging advancements in the analyses of functional, connectivity and structural data, Brühl, Delsignore, Komossa and Weidt (2014) provided an updated meta-analytic review of neuroimaging literature in SAD that had followed since the publication the earlier review, including a novel neurobiological model of SAD. Brühl et al (2014) confirmed hyperactivation of the fear circuit in SAD, especially in response to disorder-relevant tasks (e.g. emotional faces, social criticism, self-referential cognitions, exposure to scrutiny, anticipation of public speaking, social situation pictures, negative emotional voices, and phobic related words), with hyperactivation most commonly reported in the amygdala, insula, and medial and ventrolateral prefrontal cortices, and to some degree in medial parietal and occipital regions (posterior cingulate, precuneus, cuneus). This *localised* hyperactivation is also accompanied by reduced connectivity between the parietal, limbic and executive network regions. Specifically, when compared to healthy controls, SAD individuals demonstrated increased activation of the bilateral amygdala, bilateral insula, bilateral medial and ventrolateral prefrontal cortex, along with higher activation of the anterior cingulate and bilateral parietal cortex. There was also evidence (though in fewer studies) suggesting increased activation in the bilateral hippocampus and fusiform gyrus in SAD (Brühl et al., 2014). The specific brain regions of the fear circuit were all hyperactive in SAD, with activation in the amygdala also found to be associated with arousal and negative affect (Sergerie, Chochol, & Armony, 2008) and trait anxiety (Baur, Hänggi, & Jäncke, 2012). Hyperactivation of the prefrontal regions observed in SAD in the Brühl et al (2014) review have been associated with emotion regulation functions, such as

cognitive reappraisal. Fear of and sensitivity toward negative faces is suggested as evidence for heightened reactivity of the emotional system in general in SAD (Brühl et al., 2014).

Of particular interest to this thesis is the physiological (i.e. specifically the hypothalamic-pituitary-adrenal (HPA) axis) and cognitive differences in the stress response of SAD. Cognitively, those with SAD present with strong negative interpretation biases in attention, memory and imagery, particularly in the social context. These are expected to contribute to increased threat and anxiety related cognitions and increased threatening interpretations of social situations (Crisan, Vulturar, Miclea, & Miu, 2016; Heimberg & Magee, 2014; Huppert, Foa, Furr, Filip, & Mathews, 2003). Maladaptive interpretation and attention biases lead SAD individuals to misinterpret social signals as indicative of rejection, or negative evaluation, due to the negative beliefs held about the self and problematic assumptions about others' expectations of social performance (Gilboa-Schechtman et al., 2017). The negative interpretation biases are associated with the use of behaviours that contribute to the maintenance of social fears and SAD (Hirsch & Clark, 2004; Vagos & Pereira, 2012). Negative cognitions and evaluations about the self (i.e. negative self-beliefs) are highlighted as integral in the aetiology and maintenance of SAD (Wells & Clark, 1997). Distorted self-beliefs are thought to contribute to the deficits in emotion regulation (Dixon et al., 2019) and heightened emotional reactivity observed in SAD (Goldin, Manber-Ball, Werner, Heimberg, & Gross, 2009). Individuals high in social anxiety have demonstrated a greater reliance on positive emotion suppression and fewer number of positive social events experienced, with maladaptive emotion regulation strategy use demonstrated in high social anxiety (Farmer & Kashdan, 2012).

Those with SAD also commonly demonstrated increased post-event processing, defined as engagement in repetitive negative thinking following an anxiety inducing social encounter, with this cognitive process thought to maintain SAD symptoms (Gavric,

Moscovitch, Rowa, & McCabe, 2017). Those with SAD have also demonstrated increased stress sensitivity, particularly in the observed negative affect reactions to social stress (Farmer & Kashdan, 2015). Often, the affective experience of SAD is characterised by high levels of negative affect, less positive affect and positive affect expression, along with lower levels of self-efficacy and confidence (Chen, Clarke, MacLeod, & Guastella, 2012; Eisner, Johnson, & Carver, 2009; Kashdan & Roberts, 2004; Kashdan, 2007; Kashdan & Steger, 2006).

Physiologically, those with SAD have demonstrated altered reactivity to social stressors (Crisan et al., 2016). Specifically in SAD, when compared to healthy controls, differences in response to engagement with a social stressor have been observed in salivary cortisol (Furlan, DeMartinis, Schweizer, Rickels, & Lucki, 2001), plasma cortisol (Condren, O'Neill, Ryan, Barrett, & Thakore, 2002) and in cardiovascular functioning and regulation (Gaebler, Daniels, Lamke, Fydrich, & Walter, 2013; Gerlach, Wilhelm, Gruber, & Roth, 2001; Pittig, Arch, Lam, & Craske, 2013). Co-ordination between the central nervous system and endocrine systems stimulate the HPA axis stress response, which results in glucocorticoid release to manage stressors. Dysregulation in the HPA axis response has been linked to anxiety disorders (Elnazer & Baldwin, 2014), however, results of research examining differences in HPA axis functioning in SAD compared to healthy controls has been varied (Crisan et al., 2016). No current evidence supports baseline dysregulation in the HPA axis in SAD when compared to controls (Plag, Schumacher, Schmid, & Ströhle, 2013). Research examining the SAD response to an acute stressor compared to healthy controls has found evidence for similar HPA axis responding (Klumbies, Braeuer, Hoyer, & Kirschbaum, 2014; Levin et al., 1993; Martel et al., 1999), increased HPA axis responding (Condren, O'Neill, Ryan, Barrett, & Thakore, 2002; Roelofs et al., 2009) and lower levels of HPA axis response in SAD (Beaton et al., 2006; Shirotsuki et al., 2009), measured through cortisol

sampling as an indication of cortisol secretion in response to stress. The existing evidence for the physiological differences in SAD compared to healthy controls is incongruent; this in part forms the basis for the empirical studies reported in this thesis in Chapters 6 and 7 during which salivary cortisol response to social stress is examined in detail.

The above research is indicative that SAD impacts upon different functional and stress response systems in the human body. The human stress response is understood as a complex phenomenon consisting of a number of distinct cognitive, neurological, emotional, physiological and behavioural systems (Campbell & Ehlert, 2012) with a lack of synchrony in these distinct stress response systems, where each response system represents a collective of independent and varying components (Campbell & Ehlert, 2012). This thesis aimed to examine the subjective and physiological salivary cortisol systems of the acute stress response (see Chapters 6 and 7 for the empirical studies), and the need for a reliable and valid stress protocol (see Chapter 5) for inducing an acute stress response to examine stress in SAD was essential. Assessment of the four most commonly utilised stress protocols in research, including the Stroop test, cold pressor test, Trier Social Stress Test, and bicycle ergometer test, demonstrated that each of the stress protocols differentially stimulated various aspects (e.g. perceived stress and HPA axis endocrine and autonomic biomarkers of stress) of the stress response (Skoluda et al., 2015). Autonomic stress responses were most effectively evoked when a stress protocol was more physically demanding (e.g. an ergometer), while stress protocols with the central elements of uncontrollability and social-evaluative threat, were most likely to elicit HPA axis responses (Skoluda et al., 2015). The TSST elicited the highest perceived and HPA axis activity in response to stress, and the second highest autonomic response, second only to the ergometer test). These results demonstrate the TSST is a highly effective acute stress test to stimulate three common markers of the stress response, autonomic and HPA axis biomarkers and the subjective perceived stress response

(Skoluda et al., 2015), and was subsequently the protocol of choice for this thesis to induce an acute social stress response in all participants. For a detailed introduction to the TSST and a detailed guide for conducting the TSST see Chapter 5 of this thesis.

CHAPTER 3. An Introduction to Ecological Momentary Assessment and Review of the Methodology in Social Anxiety Disorder

3.1. Overview of Chapter

Dating as far back as the mid 19th century, research has explored the need for assessment methodologies that are more representative of daily life and real-world experiences in research practices, as opposed to artificial laboratory assessment (Mehl & Conner, 2012). One such research methodology is ecological momentary assessment (EMA), which involves assessment of everyday experiences in daily life (see section 3.3. Ecological momentary assessment: overview and strengths for further details). In contrast, traditional behavioural research methods saw observation and observational reports as the key source of data and were heavily reliant on the study of overt behaviours in the laboratory setting (Scollon, Prieto, & Diener, 2009). The progression into the use of traditional self-report research methods, such as retrospective and trait self-reports, gave rise to improved assessment of a range of events and a wider range of psychological phenomena. However, current methods used in the laboratory are lower in ecological validity and generalisability of results to real-world experiences. The limitations of traditional research methods will be discussed next. Then, we will provide an overview of the use of EMA including its strengths and weaknesses to study various psychological phenomena.

3.2. Limitations of Traditional Research Methods

The research methodology known as EMA[2] presents with three key advantages over traditional research methods: (i) real-world exploration of psychological functioning in response to real events in daily life, rather than in response to artificial laboratory stimuli or

[2] Ambulatory assessment (AA) is an overarching term used to encompass the increasingly computerised experience sampling method (ESM; a methodology traditionally having occurred through self-report paper and pen assessments), ecological momentary assessment (EMA; the use of electronic diaries, handheld computers or smart phone mobile devices), and psychophysiological, biological and behavioural monitoring (Trull & Ebner-Priemer, 2013). Many researchers use the terms AA, ESM, EMA interchangeably in research (Trull, Ebner-Priemer, Brown, Tomko, & Scheiderer, 2012). EMA has expanded beyond naturalistic self-reported subjective states and behaviours to also include physiological monitoring (e.g., heart rate). For clarity, consistency and relevance to this thesis, EMA will be used primarily as the descriptive term of reference hereafter.

scenarios; (ii) real-time capturing of experiences in the moment, in contrast to relying on retrospective recall or an individual's beliefs about their typical experiences; and (iii) dynamic assessment through intensive longitudinal data and its capture of processes as they unfold over time within individuals (Mehl & Conner, 2012).

EMA has grown in popularity over the previous two decades with its promise to gather ecologically valid self-report, physiological and biological data across daily life (Trull & Ebner-Priemer, 2013). Data gathered from EMA methodologies can be used to characterise and test dynamic psychological processes, including but not limited to, cognitive styles and processes, the experience of emotions, behaviour and physiology in daily life (Trull & Ebner-Priemer, 2013). The past few decades have witnessed a dramatic increase in the use of digital technology in clinical psychology research and practice. Yet, clinical assessment of psychopathology and psychological problems continue to rely largely on traditional methodologies, such as pen-and-paper questionnaires and face-to-face clinical interviews (Trull & Ebner-Priemer, 2013). While these traditional methods have their merits and are valid, they suffer from several important limitations. Traditional methods are typically reliant upon a participant's retrospective self-report, and on the ability of the clinician or researcher conducting the assessment or interviews, and are assessed within an artificial laboratory environment or clinic (Trull & Ebner-Priemer, 2013). This next section will focus on the limitations of traditional research methodologies in relation to clinical psychology.

3.2.1. The artificial laboratory environment. Traditional methods do not capture psychological functioning and symptoms in the real world, but rather in the clinic or the laboratory. While laboratory studies often report high internal validity, the ecological validity of these studies is commonly lower. The ecological validity of a study is a reflection of how accurately the study is representative of the conditions by which the effect occurs outside of

the artificial environment (Reis, 2011). Often, the internal validity of a study has been prioritised over external validity and the importance of knowing the independent variable in question is the true source of change in the dependent variable has been most valued. The consequence of prioritising internal validity means research findings cannot be generalised to other environments or samples and the potential impact of real-world conditions or contexts in modifying a process or phenomenon goes unanswered (Reis, 2011). The artificial laboratory environment and its carefully controlled conditions allow for exploration of 'what can happen' to the process being explored, rather than a focus on 'what does happen' in real-life (Reis, 2011).

3.2.2. Retrospective reporting. Traditional methods do not capture psychological functioning and symptoms in real time, but instead rely on retrospective reporting or people's beliefs about their functioning/symptoms. Retrospective reports are reconstructions of experiences – characterised by the individuals' current interpretation and are a reflection of numerous cognitive and motivational processes that effect encoding, retrieval, storage and assessment of episodic memory (Reis, 2011). Regardless of careful planning, design and execution of self-report surveys, retrospective responses to these surveys can be biased (Schwarz, 2007), and the results are often susceptible to being grossly exaggerated and significantly influenced by the immediate context and current state of the individual at the time of record (Fredrickson, 2000). Moreover, individual responses to self-report questions are largely influenced by the limits of human memory and recall (Tourangeau, 1999). While neither right nor wrong, retrospective reporting provides a specific type of information. If a researcher is concerned with how an individual experiences or understands events in their life following a period of reflection, then retrospective reports have the potential to inform understanding of transformational and reinterpretative processes. However, if a researcher is interested in what actually happened, devoid of recall biases and memory heuristics, then

traditional retrospective reporting is limited in its ability to circumvent these processes (Reis, 2011).

3.2.3. Static capture. A major limitation of traditional methods that is often forgotten, is that it does not capture symptomology and functioning as dynamical processes that fluctuate over time. The experience of feelings is subjective, and access to these feelings and experiences is the privileged information of the individual having them. While not always apparent or easily identifiable for the individual reporting, research typically assumes the individual is the final arbiter of these feelings or experiences (Schwarz, 2011). However, evidence suggests that while the individual reporting is the greatest informant of their own experience, what they do report regarding their feelings and experiences is likely to change according to their different interpretations at different times. This is supported by a Schwarz (2011) review of individual access to emotions for self-report, which documented that profound differences occurred between an individual's concurrent and retrospective reports on emotion and experience. Traditional research methods have often assumed psychological processes to be static and relatively unchanging, focusing on the already existing *product* rather than *processes* (Lidz, 1991). Single-occasion assessments of dynamic psychological processes and psychopathology are unable to characterise how the ebb and flow of behaviours, mood, symptomology and cognitions changes across contexts and time (Trull, Ebner-Priemer, Brown, Tomko, & Scheiderer, 2011). As a rule of thumb, trait or global measures tap into increasingly deliberate, reflective and stable knowledge (Conner & Barrett, 2012). In light of this, applying trait or global measures as a short-cut to understanding the experiencing self (e.g., momentary consciousness) can result in reduced sensitivity in measurement, smaller effect sizes and even null results (Conner & Barrett, 2012). The danger of traditional methods is to use static, retrospective or trait assessments as proxies for dynamic real-life experiences. Relationships between experiences, memories and traits may

be observed, however exploring these in a static manner will not necessarily demonstrate the associations that exist with a predictor or criterion of interest, nor will they reflect the impact of context and changes in time (Conner & Barrett, 2012).

3.3. Ecological Momentary Assessment: Overview and Strengths

EMA affords the ability to collect particularly rich descriptive data, providing several conceptual advantages when compared to a traditional research design. Traditional designs have seen researchers frequently assume psychopathology symptoms are not particularly dynamic but rather fairly static, having characterised complex and frequently changing psychological processes through single-occasion assessments or observation (Trull & Ebner-Priemer, 2013). The three primary ways in which EMA overcomes limitations of traditional methods is by allowing researchers to study psychological processes in (a) the real world; (b) in real time; and (c) dynamically. This section will discuss each of these main strengths of EMA, in turn.

3.3.1. Real world – Outside of the artificial laboratory. Unlike research conducted in the laboratory, EMA methodologies see processes of interest studied in the natural habitat of an individual and therefore maximise ecological validity. These processes are explored within the environmental and interpersonal factors that are typical to the individual's experience, which cannot easily be recreated in the lab. Additionally, EMA also allows researchers to sample the defining characteristic of the environment (e.g., location and time of day) in which a psychological process occurs. The environmental characteristics can help to explain changes in the psychological process being observed (Trull & Ebner-Priemer, 2013), such as complex behavioural contingencies. For example, EMA can be used to study how fluctuations in momentary affect influence particular behaviours over time. This provides a better understanding of the co-occurring role of both the person and the situation

giving rise to various psychological processes and experiences (Scollon et al., 2009). The capture of life as it is lived, in terms of description of behaviours as they occur spontaneously in the naturally occurring context, provides extensive data examining social, psychological and physiological processes as they unfold across daily life, which is unobtainable from lab assessments. The premise of EMA is that the context in which the above processes occur influences behaviour and contextual factors, and therefore must be taken into account – via real world sampling (Reis, 2011).

3.3.2. Real time – Avoiding retrospective reporting. Traditional self-report methods are valid, however when compared to EMA, they capture different sources of information. In contrast to these traditional investigations that rely on retrospective self-reports (e.g., how anxious have you felt over the past two weeks?) or global/trait self-reports (e.g., how anxious do you typically feel?), EMA studies are advantageous in their design to capture momentary ratings which attempts to gather data in real-time or in close proximity to real-time (e.g., right now, how anxious do you feel?) (Conner & Barrett, 2012). The accuracy of the EMA reports has been shown to be higher than that of retrospective reports of occurrence of events, behaviours and experiences (Trull & Ebner-Priemer, 2013), which highlights the incremental validity of EMA over retrospective and dispositional reports of an individual. If the desired aim of the research is to understand an individual's momentary emotional responses to particular events, EMA is most valid. Trull and Ebner-Priemer (2013) further emphasise that if the goal is to assess biological, psychological or behavioural processes, then the need for multiple assessments across a relevant time period is particularly important. EMA is uniquely qualified to provide time contingent measurement of biological and psychological experiences in the real world.

An additional strength of EMA has come from the computerisation of the EMA methodology. This has facilitated capture of data in real-time more easily through the added

convenience of electronic devices and the time-stamping of the electronic responses. The use of electronic devices has shown that participants typically exhibit higher rates of compliance in reporting at the time of signal, with a wide range of clinical populations shown to provide timely responses for greater than 85% of delivered signals (see Hufford, Shields, Shiffman, Paty, & Balabanis, 2002; Stone & Shiffman, 2002; Trull et al., 2008).

Arguably one of the greatest promises of EMA is the methodology's ability to reduce memory biases. Bias problems for self-reports include biases in retrospective recall (i.e., intentional or unintentional differential recall of information); autobiographical memory (i.e., a combination of episodic and semantic memory framing recall around personal experience), or the use of heuristics in response patterns (i.e., using rules/mental shortcuts to make judgments or decisions). Traditional self-report formats are reliant on memory-based reporting where individuals recall and summarise their experiences, often over a long course of time including weeks, months or even years (Conner & Barrett, 2012). EMA provides a means to circumvent self-report biases due largely to the closeness of proximity between responses to the occurrence of the event or situation in question (Conner & Barrett, 2012). As these reports attempt to characterise the current experiencing self, the reports are more likely associated with physiological and biological processes of the states that have been reported and not those of retrospective or trait self-reports (Conner & Barrett, 2012).

3.3.3. Dynamics. Despite the understanding of psychological processes unfolding over time, research has historically explored these processes in a static manner. Research has often used cross-sectional data and large sample approaches, and operated under the assumption that the differences observed between individuals are reflective of the within-person processes (Hamaker & Wichers, 2017). The capacity for cross-sectional and large-sample approaches to provide information on the processes that take place at the within-person level is limited, establishing a need for alternative approaches (Hamaker, 2011).

A major strength of EMA is its ability to encompass both nomothetic (e.g., large samples, generalised population results) and idiographic investigations (e.g., study of a particular individual) (Hamaker, 2011). EMA allows for within-person analyses at the level of the unique individual, to accompany the previously largely dominant between-person analyses. No longer limited to between-person explorations of psychological phenomena, within-person sampling can allow for interesting, and sometimes unexpected patterns or novel insights to emerge that may otherwise have been hidden at the mean level (e.g., population results) (Scollon et al., 2009). The data collected through EMA is able to characterise dynamic psychological processes including affect, cognitive styles and expectations, patterns of behaviour and accompanying physiological experiences across daily life (Trull & Ebner-Priemer, 2014). The ability to explore individual variations and differences in regard to the intensity of psychological experiences that occur across time and place, is paramount to EMA's value in research.

Recent times have seen an exponential increase in the use of intensive longitudinal data obtained via EMA methods. The beauty of intensive longitudinal data lies in its ability to allow for insights in to the dynamics of a process, seen in the manner by which external and internal forces impact the course of the phenomenon being explored or assessed (Mehl & Conner, 2012). Such temporal dynamics can be modelled with intensive longitudinal data using time-series analysis or dynamic multilevel modelling. Time-series analysis has been developed within econometrics, physics and engineering, but has rarely been applied to the field of social sciences (Hamaker & Dolan, 2009). The analyses is a single-subject technique, whereby a large amount of repeated measures produced from a single system are analysed. The focus lies on the current observation and how it may be predicted from previous observations of the same and/or alternative variables. More recently, dynamic multilevel modelling has been developed. Multilevel modelling is based on a time-series model at

Level-1, which describes within-person processes, with Level-2 modelling the between-person difference in the dynamic features (Hamaker & Wichers, 2017).

The breadth of modern research reflects a paradigm shift within psychological research, moving away from attempts to understand dynamic psychological processes through static outcomes and cross-sectional reports, towards an analysis that reflects investigation of both the within-person dynamics of psychological process and the between-person differences therein (Hamaker & Wichers, 2017). The aforementioned statistical advances have facilitated working with intensive longitudinal data and provide the ability to tackle within-person questions and overcome prior statistical methodological barriers that previously hindered exploration of processes at the within-person level (Hamaker, 2011). While the emergence of intensive longitudinal data and the statistical techniques to work with such data provides significant advancement for research into psychological processes in novel and innovative ways, it also brings along with it a number of methodological and statistical challenges (Mehl & Conner, 2012).

Statistically, it is imperative that within-person dynamics are separated from between-person differences so that results are informative of the within-person process, and not a blend of within-person and between-person relationships that cannot be interpreted. It is also important to control for between-person, stable, trait-like differences while also accounting for moment-to-moment stability, before considering external predictors. If these two forms of stability are unaccounted for, within-person associations between predictors may be distorted (Hamaker & Wichers, 2017). Statistical analysis should also acknowledge the use of a random interval design, which does not fit neatly into a time-series or multilevel model, both of which are typically based on a fixed or equal interval assumption. Future research is needed to determine the cost of ignoring unequal intervals in analysis, along with investigating the optimal time grid to be used if attempting to make the observations equally

spaced. Awareness of the nature of time, the function of lagged relationships between variables, and deciding whether a process should be considered continuous or as occurring at particular points in time is essential for researchers in the statistical analysis of intensive longitudinal data (Hamaker & Wichers, 2017).

3.4. Weaknesses and Pitfalls of Ecological Momentary Assessment

While EMA has many strengths, those employing the methodology must also understand and consider the potential pitfalls. The concerns associated with EMA can be categorised as issues with participants, representative sampling, measurement, and data analysis (Scollon et al., 2009). This next section will discuss some of the weaknesses and potential pitfalls to be considered when using an EMA methodology.

3.4.1.Participant burden and self-selection. The often-demanding nature of EMA raises concerns with self-selection biases and participant attrition. The significant participant burden of an EMA protocol, such that they often prompt a participant with alarms up to 10-12 time per day across 1-2 weeks, can be disruptive and demanding for the participant. The interruptive nature of the survey signals may be particularly unsuited to groups of individuals who are more time poor (e.g., full-time professionals with children) and who will require greater motivation, compared to groups who may have less daily life demands (e.g., college students or the unemployed) who will require less motivation to attend to signals (Scollon et al., 2009). Participants may also be deterred from participation due to the perceived complexity of the protocol or demands of modern technology (both the tools, and operating software). This may result in those with greater motivation or openness to technology only taking part (Palmier-Claus et al., 2011) resulting in a biased sample of participants.

Research has shown that the motivation of a participant plays a large part in the completion of an EMA protocol. The intrinsic motivation, agreeableness or general

conscientiousness of a participant may see the successful completion of an EMA protocol. However, those less motivated may drop out after a few days, while some individuals may all out refuse to participate. Thus, the potential for over or under representation of certain characteristics within a population can be problematic for EMA, and researchers must be cautious with generalisations made (Scollon et al., 2009). Further, the suitability of EMA for certain groups, for example, the elderly or children, those unfamiliar with technology, those unable to hear to the signal notifications, those unable to view the screens of devices used for EMA or those for who it would be unsafe to attend to a signal (e.g. drivers) can pose a challenge. Those most likely to show the greatest compliance within an EMA study are conscientious, young, agreeable individuals who are not experiencing any depression and are not overly time-poor. As such the generalisability of the results must be considered carefully in EMA studies, and the applicability of the "daily life" experiences captured by the sample to the general population should be explored (Scollon et al., 2009).

3.4.2. Clinical participants. Many researchers have demonstrated hesitancy or scepticism toward severely disordered participants' ability to comply with the demands or extensive nature of EMA protocols. However, this scepticism is largely unfounded, with severely ill participants, such as those with borderline personality disorder, substance dependence, or schizophrenia, demonstrating good compliance and low attrition (Trull & Ebner-Priemer, 2013). Further, evidence of censorship of self-reports (e.g. providing more desirable responses or incomplete truths) has not appeared in the reports of severely ill participants, with endorsement of dysfunctional or undesirable social behaviours occurring (Trull & Ebner-Priemer, 2013).

3.4.3. Reimbursement. The burdensome nature of most EMA studies suggests participant reimbursement should be generous. Participant compliance in EMA studies is shown to significantly improve with monetary incentives (Lynn, 2001). However, the nature

of reimbursement can be problematic, with larger sums of reimbursement potentially incentivising participants in a way that is harmful to the research and data quality (Dickert & Grady, 2008). The impact of participant remuneration on the individual's decision to participate in research must be considered in the context of how incentives to participate may influence participant motivation (Dickert & Grady, 2008). Several factors for consideration when determining an appropriate participant reimbursement sums include: Commodification (e.g. the participant becoming a commodity of research), justice (e.g. equal distribution of benefits and burdens of the research participation), incentivising risk (e.g. agreeing to higher risk due to a higher monetary incentive), exploitation (e.g. taking advantage of individuals of lower socioeconomic status), intrinsic motivation (e.g. care for end result of the research), and the possible influence of greater remuneration sums on concealment of information by participants to prevent exclusion from the research (Dickert & Grady, 2008).

3.4.4. Participant training. The complex nature of EMA methodologies often requires additional time demands and training of participants. Participants need to be well-versed in the procedures and tasks they are required to follow and have a thorough introduction to and training for the use of the testing equipment involved. To contribute to participant compliance and motivation, participants should also be well-versed in the protocol, the importance of the study they are participating in and their contribution to the research (Shiffman, Stone, & Hufford, 2008)

3.4.5. Select sampling of situations. Another potential concern of EMA is the sampling of select situations. Random sampling of a person's everyday life opens a whole host of potential sampling concerns with the participant choosing whether to answer a signal or not. Some instances have a greater likelihood of participants choosing not to answer (e.g. when engaged in a task), other circumstance make it impossible to answer (e.g. while driving) and some signals may be received when a disruption is not appropriate (e.g. work

meetings or church). The great ecological strength of EMA is the ability to capture a full range of participant activities and daily situations, requiring participants to answer signals even when inconvenient or when they feel unmotivated to do so. As such, this emphasises the need for researchers to convey the importance of answering signals across all situations and the provision of clear and explicit protocol instructions to participants (Scollon et al., 2009).

3.4.6. Reactivity. Another potential limitation of EMA is measurement reactivity, or the potential for psychological process under investigation to change as a result of being studied (Wheeler & Reis, 1991). Reactivity is a challenge considered in all research into human behaviour, however, it is of particular interest in EMA where repeated assessment potentially impacts the degree to which participants pay attention to their internal experiences or behaviours. In addition to priming participants to be more attune or aware of their experience or states, the intrusive nature of EMA may contribute to participant irritation or burden. It has been demonstrated however, that the use of EMA did not lead to increases in negative mood due to the intrusive nature of the signals (Cerin, Szabo, & Williams, 2001). Research has shown comparatively to repeated or retrospective measures, EMA measures of cognitive intrusion were lower (Scollon et al., 2009). It is noted a continue concern for EMA is that increased attention toward one's anxiety or worry has the potential to trigger increased cognition or rumination around these anxieties that can in turn lead to increases in these states (e.g. Mathews & MacLeod, 1994).

Barta, Tennen and Litt (2012) explored the threat of reactivity as a concern for EMA self-reports. While in general, it has been demonstrated EMA self-reports are not affected by reactivity, there has been such an effect observed in some studies. Conner and Reid (2012) reported reactivity effects on the reporting of mood when using mobile technology. Courvoisier, Eid and Lischetzke (2012) also reported evidence of reactivity in a time-dependence compliance pattern. Efforts to detect reactivity or to protect against potential

reactivity have been suggested by Barta et al (2012), such as, through use of appropriate control groups (e.g. non EMA use), examination of data to note any trends or response changes that could underlie reactivity effects, noting any changes in responses that suggest a participant may have changed the meaning assigned to assessment scales, assuring that data is captured or contributed to on days or times that may be associated with understanding the behaviour of interest (e.g. collection on weekends or evenings, times commonly associated with consumption of alcohol; Trull & Ebner-Priemer, 2013). It is however for future EMA research to continue to explore whether EMA is in fact measuring a phenomenon as is, or the phenomenon transformed by measurement itself and further, whether reactivity differs across the different groups and variables measured through EMA (Scollon et al., 2009).

Further, due consideration must be given to the potential limitations of self-report in general, and researchers should aim to explore and navigate these limitations carefully. While EMA aims to eliminate retrospective bias, there remains the potential for the limitations of self-report such as social desirability, cognitive biases and cultural norms to continue to influence responses. While self-report serves as the only method to truly capture the subjective experience of an individual and the method is essential to psychological research, possible remedies for these limitations should be explored (Scollon et al., 2009).

3.4.7. Data quality. The quality of the data is also a potential pitfall of EMA if not considered carefully. Declining quality of data is estimated to occur after a 2-4-week period of sampling, with paper and pencil tests also prone to participants fabricating their responses or completing all forms on one occasion. While this can be addressed by having participants hand in forms daily, digital devices can reduce fabricated responses by applying a date and time stamp to completed signals or an expiry time-frame, which highlights clearly if a participant has attempted to complete all reports in one sitting or missed responses. Consideration should also be paid to the potential for participants to use the same responses

repeatedly, becoming habitual in answering. However, similar answers across reports could also be reflective of greater accuracy or self-awareness. With the difficulty of differentiating between greater accuracy and self-awareness or potentially habitual responses, weighting procedures (as detailed by Stone, Kessler, & Haythomthwatte, 1991) can help to correct for habitual responses. Further, the careful piloting and refining of proposed procedures and assessments (see Reis & Gable, 2000) can reduce the issue (Scollon et al., 2009).

3.5. The Introduction of Smartphones

The past decade has seen a surge of EMA research as smartphone ownership has become increasingly widespread. As reported in the Deloitte Mobile Consumer Survey 2018 (Deloitte, 2018), current Australian smartphone ownership sits around 89% with smartphone penetration expected to exceed 90% in 2019. The capabilities of modern smartphones, such as the capacity to operate built-in sensors (e.g., accelerometers), link with external sensors (e.g., cardiac activity, electro-dermal activity), employ GPS capabilities and record video, have seen smartphones become the central core of EMA. Conducting EMA research using apps installed on participants' own smartphones presents with several advantages, such as increased convenience, rapid collection of large and diverse samples, dramatically reduced equipment costs and a more natural fit to participants natural environment (Thai & Page-Gould, 2018). A large number of consumer electronic applications or 'apps' are at present available for individuals to monitor their behaviours, habits and symptoms – making it apparent that individuals have a keen interest in exploring or improving their experience via their smartphone device. Luxton, McCann, Bush, Mishkind and Reger (2011) completed a search for health and mental health related apps and in turn discovered several hundred apps available across a range of health topics such as anxiety, depression, nutrition, alcohol use,

sleep and general well-being. It stands to reason, that the current climate of individuals often having a smartphone device readily accessible or in use, along with the apparent interest in exploring or recording their health experience, presents a unique opportunity to use this within research into psychopathology.

3.6. Using EMA in Clinical Psychology: A Focus on Social Anxiety Disorder

The use of EMA presents a unique opportunity to provide an in-depth account and rich understanding of clinical problems as they unfold in daily life, with the potential to improve clinical diagnosis and intervention. EMA is currently used in the investigation of the mechanisms and dynamics of psychopathology and its symptomology, prediction of reoccurrence or symptom onset, prediction of treatment success and relapse prevention. Further, EMA is used to provide interventions in real-time and monitor treatment effects, progress and outcomes. The methodology has been employed broadly across psychological assessment and in the assessment of clinical problems. For example, an entire specialty section on the use of EMA in clinical assessment was published in Psychological Assessment in 2009, which included publications that focused on substance use and substance use disorders (Shiffman, 2009), anxiety disorders (Alpers, 2009), mood dysregulation and mood disorders (Ebner-Priemer & Trull, 2009), and psychosis (Oorschot, Kwapil, Delespaul, & Myin-Germeys, 2009). In addition, several reviews exist for the use of EMA in specific clinical disorders (e.g. depression, Telford, McCarthy-Jones, Corcoran, & Rowse, 2012; Anxiety disorders, Walz et al., 2014).

The repeated nature of EMA sampling has enabled research to investigate not only how symptoms of psychopathology are related to each other across various intervals and environments, but also how symptoms fluctuate over time. This has allowed for a far deeper understanding of the mechanisms involved and the dynamic nature of psychopathology.

Traditional clinical assessments are unable to explore or characterise the distinctive ebb and flow of psychopathological symptoms and many disorders and symptoms are characterised by instability and an ever-changing landscape. Several forms of psychopathology, such as depression and anxiety, are characterised by increased intensity of mood states and the potential for extreme fluctuations in these states (Trull et al., 2012). Such features can only be measured across multiple and frequent assessments in order to capture the variability that exists within these disorders and symptoms (Trull & Ebner-Priemer, 2013). Of particular interest to this thesis is the psychopathology of social anxiety disorder, with this typically involving elevations in fear, threat reactivity, distress and panic (Trull et al., 2012).

3.7. EMA and Social Anxiety Disorder

The use of EMA to research and assess the dynamic nature of SAD symptomology is particularly useful, with the symptoms of SAD often being unpredictable (such as panic attacks) and restricted to specific situations (such as social events). The profile of SAD is often context dependent (i.e. fear or anxiety is related to the social context) and fluctuates across time. The socially based fear or anxiety experienced can also occur in isolation, meaning with or without direct social engagement or interaction. The assessment of the frequency, intensity and duration of anxiety symptoms helps to distinguish those with SAD from a typically healthy experience, as the symptoms of social anxiety are known to occur with greater frequency and to a far higher extent and duration in SAD than in control populations (Kashdan et al., 2013). The widely varying profile of panic, fear and anxiety symptoms seen in SAD can significantly increase the difficulties and challenges faced when attempting to assess SAD and its related constructs within a laboratory setting or via retrospective subjective self-reports (Walz et al., 2014). EMA can overcome these challenges by yielding momentary real-life assessments of social anxiety symptoms through reporting

that is close in time to the naturally occurring context of these symptoms, reducing memory biases and increasing not only the generalisability and ecological validity of the data but contributing to the understanding of how these may change across different times and contexts (Kashdan & Farmer, 2014). Despite the knowledge that EMA may offer valuable contributions to the understanding of the disorder and a novel way to explore the psychopathology and real-world experiences of SAD, only twelve EMA studies on SAD have been published, of which five were reviewed by Walz et al (2014) in their systematic review of existing anxiety disorder literature (see Faytout & Swendsen, 2009; Johnson et al., 2009; Kashdan & Farmer, 2014; Kashdan et al., 2014; Russell et al., 2011). Seven further publications exist that use EMA toward the understanding and assessment of SAD (see Farmer & Kashdan, 2014, 2015; Gloster et al., 2017; Goodman & Kashdan, 2019; Nylocks, Rafaeli, Bar-Kalifa, Flynn, & Coifman, 2018; Villanueva et al., 2019; Wilson, Koerner, & Antony, 2018). These studies have contributed to a far richer picture of the dynamic nature of SAD in real life, contributing to the research and understanding of the phenomenology of SAD in an innovative and novel way. The next section will focus on a brief summary of these studies.

Faytout and Swendsen (2009) prospectively examined the routine daily behaviours and emotions characteristic of individuals with SAD and compared these to typically healthy controls. The study protocol involved participants answering questions regarding activities, social contact and emotions via portable computers for a duration of one week. The study found that while the frequency of social interactions was similar between the SAD and control group, those with SAD reported increased interactions with family members and fewer interactions with friends. Furthermore, while individuals with SAD reported higher levels of negative affect than controls overall, individuals with SAD unexpectedly experienced a mood-enhancing effect of social interactions. These results indicate that while

the frequency of interactions was similar between groups, individuals with SAD may opt to avoid social interactions outside of the family context.

Much like the frequency findings above, Russell et al (2011) found those with SAD reported increased interactions with romantic partners when compared to controls. Further, Russell et all (2011) utilised event-contingent recording to study interpersonal encounters. The results reported that when compared to controls, those with SAD exhibited greater socio-behavioural differences (e.g. submissive behaviour) when experiencing increased feelings of anxiety or emotional insecurity in contrast to when they were not experiencing increased feelings of anxiety and insecurity. For example, those with SAD were reported as being more submissive when anxious, and more agreeable and less quarrelsome when insecure.

A combination of event-contingent recordings of social interactions, with signal and time recording of the emotional experience of those with SAD was employed by both Kashdan and Farmer (2014) and Kashdan et al. (2014). Specifically, Kashdan and Farmer (2014) focused on the rating of social interactions in regard to positive and negative affect and the ability of those with SAD to differentiate between positive and negative emotional experiences. Those will SAD were less able to differentiate negative emotions and as expected, recorded increased levels of social anxiety and situation avoidance when compared to controls. To further distinguish those with SAD from the control group, SAD individuals recorded decreased intensity and frequency of positive emotions and this was accompanied by the avoidance of anxious feelings, highlighting dysfunctional experiences of both negative and positive affect. Participants in Kashdan et al.'s (2014) study rated the anxiety and avoidance that accompanied social interactions. This study demonstrated that the tendency to avoid internal undesired thoughts and feelings (i.e. momentary experiential avoidance) was positively correlated with symptoms of anxiety experienced during social interactions, an effect that was significantly higher among SAD individuals than controls. In sum, the Walz et

al (2014) systematic review suggest that when compared to controls, those with SAD differed in the type (rather than frequency) of the social interactions, their social behaviours were likely to change only when feeling increasingly anxious or emotionally insecure, and problems differentiating negative, but not positive, emotions were apparent.

In a study assessing end of day reports of affect and self-esteem in SAD, Farmer and Kashdan (2014) explored instability in these measures in daily life and aimed to develop an improved understanding of the temporal fluctuations of affect and self-esteem in daily life in SAD. When compared to healthy adults, SAD individuals experienced increased and less stable negative affect, and lower but stable (i.e. less likelihood for acute shifts) positive affect across daily life. These findings suggest that individuals with SAD demonstrated increased instability in negative affect, with greater likelihood of acute moment-to-moment shifts in negative affective states (but not positive), as well as difficulty in recovering from negative affect and maintaining positive affective states (i.e. problematic self-regulation)(Farmer & Kashdan, 2014). To our knowledge, Farmer and Kashdan (2014) is the only study to date that has used EMA to explore self-esteem experiences in SAD across time, despite models of SAD highlighting the role of reduced and changeable self-esteem in the phenomenology of the disorder (Clark & Wells, 1995; Moscovitch, 2009). Farmer and Kashdan (2014) add support to global reports of lowered self-esteem in SAD and found those with the disorder displayed greater instability in self-esteem, however, this instability was driven by mean level differences in self-esteem and not apparent when controlling for mean levels of self-esteem. This study highlights not only the presence of increased negative affect, lower self-esteem and attenuated positive affect (i.e. positivity deficits) in SAD, but further the presence of instability in negative affect and self-esteem (based on mean levels). Instability in affect potentially leads to feelings of uncontrollability and therefore an increasingly threatening experience. The increased experience of threat interpreted from instability in negative affect

may contribute to attempts to supress, conceal, or avoid these emotions altogether, with these kinds of emotion regulation strategies associated with maintenance of the disorder.

Farmer and Kashdan (2015) examined how strongly those with SAD react to social stress in daily life, specifically they reported on stress sensitivity and stress generation in SAD. Through assessment of both the immediate and following day effects of positive and negative social engagement using EMA techniques, they found that in response to social stress in daily life those with SAD responded more strongly in their negative affect responses compared to healthy controls. Further, those with SAD reported interpersonal stress at an increased frequency when compared to healthy controls. In line with their predictions for stress generation in SAD, those with the disorder reported negative social events more often, along with reduced reporting of meaningful positive social events in their every day to day experience. An interesting finding by Farmer and Kashdan (2015) was the absence of evidence supporting an increased likelihood for social stress to occur in SAD individuals following the experience of intense negative affect in the days prior. The study concluded evidence for greater stress sensitivity in SAD in daily life, increased rigidity in reactions (i.e. consistently high stress sensitivity) and greater reports of stressful experiences in the disorder compared to healthy controls (Farmer & Kashdan, 2015) and sheds light on the specific heightened stress sensitivity toward social engagement in SAD, increased generation of stress in SAD and the increased negative and decreased positive experiences common to SAD.

Gloster et al (2017) explored the poorly understood symptom fluctuations and the dynamic contexts that provoke these fluctuations in symptoms across the clinical disorders of MDD and SAD, compared to controls. The study aimed to rigorously illustrate and test the proximal environmental, neurobiological and psychological factors that are associated with the symptomology of these disorders and mood states. The EMA component of the study was administered for one week via smartphone with assessments occurring six times per day and

querying emotions, symptoms and occurrence of daily events, social interactions and well-being. Physiological measurements such as cortisol and actigraphy were also collected during the EMA component. The intensive longitudinal data collected by this study will allow for in depth examination of symptom fluctuations and contribute to the understanding of the psychopathology of two highly prevalent disorders, along with general well-being within these clinical presentations. This paper was a design and methodological paper describing the assessment of a number of factors utilising EMA techniques and reported on the merits of a multi-modal design, without reporting results and with no specific research question addressed.

Wilson et al (2018) examined positive feedback seeking (or excessive reassurance seeking) and negative feedback seeking in SAD and GAD, compared to controls. A daily diary method was employed across two weeks to explore group differences in frequency of, topics for, and targets (e.g. family, romantic partners) of, feedback seeking (both positive and negative). Those with SAD and GAD demonstrated significantly higher feedback seeking when compared to healthy controls, with the most common targets of feedback seeking being other people (e.g. family, romantic partners). However, after adjusting for the self-reported compliance of diary completion and applying Bonferroni correction, there were no significant group differences in the frequency of positive, negative and overall feedback seeking.

Nylocks et al (2018) explored the influence of negative and positive emotion on future health-promoting behaviours in individuals diagnosed with MDD and/or SAD and healthy controls. Adaptive health behaviours, such as exercise, are shown to provide a range of health benefits, though knowledge of how emotions may influence or precede these adaptive behaviours is sparse. Nylocks et al (2018) had participants complete a two-week EMA diary measuring within-person fluctuations in positive and negative emotion and health behaviours to examine the role of within-person fluctuations of negative and positive affect

on future health behaviours. Results determined within-person levels of positive emotions were significantly associated with positive future adaptive health behaviours, with mean positive affect also associated with individual engagement in positive health behaviours. No group differences were apparent, nor were there any significant associations for within-person or mean negative affect. The results suggest increases in within-person positive affect can predict future reports of positive adaptive health behaviours.

Goodman and Kashdan (2019) examined the life goals of those with SAD and the extent to which those with SAD found their anxiety and suffering a barrier to the pursuit of personal goals and an impediment to finding meaning in life. With threat avoidance taking precedent over pursuing rewarding experiences in SAD, there is potential for those with the disorder to derive less reward from their external surrounds and life experiences. The study explored whether the experience of anxiety and suffering in SAD was associated with the decreased pursuit of meaningful life goals. Results demonstrated those with SAD viewed their anxiety and suffering as a greater interference to achieving personal goals compared to healthy controls. The study suggests the pronounced negative beliefs held around anxiety and suffering in SAD may impede those with the disorder deriving meaning in their life.

A recent Villanueva et al (2019) study examining technological social interactions (e.g. phone and internet/chat) and face-to-face social engagement in SAD, MDD and healthy controls found that healthy controls were frequently engaged in face-to-face social engagement, while those with SAD engaged with phone-based social engagement more often. Further, across the entire group, the was a positive relationship between negative affect and technology based social engagement, and a positive relationship between face-to-face social engagement and positive affect. This positive mood enhancing effect of face-to-face engagement is similar to the positive mood influence Faytout and Swendson (2009) found from in-person social interaction in SAD. Villanueva et al (2019) raise an interesting point

for consideration when planning for the use of technological based assessment and intervention, being that the use of technology based social interaction or engagement is associated with increased negative affect. Given the negative affect observed from technology based social interaction, as opposed to the positive affect observed from face-to-face engagement, future research should be mindful of the potential negative impact of technological based assessment on outcomes observed through this form of assessment. Further, with the associations reported by Villanueva et al (2019) between positive affect and social engagement and the propensity for positive mood enhancing with face-to-face interaction, future treatment and social engagement planning in SAD should be mindful of the potential benefits of face-to-face engagement in those with the disorder.

In sum, SAD individuals do not limit the frequency of their social interactions, however, a great number of interactions with family and romantic partners and reduced interactions with friends are observed. Greater situational avoidance and avoidance of anxious feelings occurred in those with SAD, though surprisingly mood-enhancing effects were experienced when social interaction did occur. SAD individuals engage in phone based social interaction at a greater frequency than face-to-face engagement, though positive mood enhancing effects are observed from face-to-face interactions as opposed to increased negative affect observed from technology-based social interaction. Those with SAD also experience decreased positive and increased negative emotions, with difficulty differentiating negative emotions also observed. Instability in negative affect, but not positive, has been demonstrated in SAD, along with instability in self-esteem (although based on mean levels). Further, changes in social behaviour (such as submissiveness or agreeableness) occurred when SAD individuals experienced increased anxiety and emotional insecurity. Stress sensitivity and stress generation was higher in those with the disorder, with SAD individuals reporting interpersonal stress at an increased frequency compared to healthy controls. Current

research also suggests positive health behaviours in SAD are potentially decreased as a consequence of the increased negative affect and decreased positive affect observed in SAD. Lastly, those with SAD found that their experience of anxiety and pain was a barrier to their pursuit of personal goals and finding meaning in their life.

Despite EMA presenting an innovative and novel way to explore psychopathology, few studies have used EMA to study SAD. With the symptomology of SAD being dynamic across contexts, time and events it seems apparent that EMA provides a unique way to begin to garner a greater depth of understanding and knowledge of the course and behaviour of the disorder. There is a dearth of research exploring SAD outside of the laboratory and a lack of understanding toward the true experience of the disorder in daily life. Future research exploring SAD through a different lens, outside of the artificial lab environment and across different contexts is required to better inform the understanding and subsequent treatment of this highly prevalent disorder. However, the aforementioned studies of EMA in SAD are not without limitations. For example, limitations include the use of end of day reporting (i.e. less time-contingent reports and reduced sampling) that is more limited in its ability to examine sources of day-to-day fluctuations and more complex sequences across time, along with different methods for operationalising psychological phenomenon (e.g. emotion regulation) across studies that may contribute to differential findings. In particular, one key limitation of these studies is that the assessment of social situations or engagement experienced across each day is different between individual participants and groups (e.g. SAD vs. healthy controls). It is difficult to capture how individuals with SAD respond to social stress using a traditional EMA design, due to avoidance of such social engagements in daily life common to SAD. The current thesis sets out to address this limitation. As we wanted to directly examine SAD symptomology in response to environmental stressors and we were unable to guarantee these would occur organically during a participant's data collection period, we incorporate a

social engagement during participation to ensure a social stressor is incurred. Specifically, while all participants in the current thesis completed an 8-day EMA protocol, we designed it such that all participants completed a laboratory based standardised acute social stress test, the TSST (Kirschbaum et al., 1993). In doing so we aimed to capitalise on the optimal experimental control of laboratory based research by having participants engage in a *lab-based acute social stress task* on day 5 of their participation, while they were using EMA to assess daily life experience.

3.8. Conclusion

EMA offers researchers unprecedented access to the daily lives of individuals. If researchers pay due consideration to the management of potential pitfalls and take precautions to avoid these where possible, EMA can contribute enormously to modern research. EMA plays an important role in understanding everyday experiences of individuals with psychopathology and could be particularly suited to the profile of SAD. EMA provides an opportunity to explore both the immediate experience of social engagement and the broader profile of anticipation and recovery from social interaction. The large scale empirical paper of this thesis (Chapter 7) reports results of the EMA protocol where all participants (SAD and healthy controls) completed ambulatory assessment across seven days, with an acute stress protocol embedded within the protocol. Daily experiences were measured at baseline, in anticipation of, and recovery from the TSST. Next, details will be provided of the methods employed; see Figure 4.2 for an overview of the thesis protocol.

CHAPTER 4. General Experimental Methods

The following chapter will provide an outline of the general methodology used to establish this thesis. The specific methodological details for the acute stress protocol conducted for this thesis is found in Chapter 5. The specific methodological details for the two empirical studies of this thesis are found in their respective chapters (Chapter 6 and 7). The following chapter will firstly detail general study information, including ethics approval and recruitment. Participant information for clinical assessment is outlined, including the inclusion/exclusion criteria, screening procedures, diagnostic assessment and clinical assessment measures used. A detailed section on the administration of the overall protocol is provided, followed by details of the statistical data packages used for data analysis. Finally, overall study population demographics and clinical characterisation measures are reported.

4.1. General Study Information

4.1.1. Ethics approval. This thesis protocol was approved by the Australian Catholic University Human Research Ethics Committee (HREC). This thesis protocol was also subject to auditing by the Australian Catholic University HREC and deemed compliant.

4.1.2. Recruitment. Participants were recruited predominantly from community-based advertising by means of web-based Gumtree advertisements, flyer distribution across multiple university campuses (e.g. Swinburne, ACU and La Trobe) and across local suburbs (e.g. Fitzroy, Melbourne CBD) and advertising on the Anxiety Disorders Australian Victoria (ADAVIC) website. Approximately one third of the control participants in this study were participants who had previously completed phases of the larger neuroimaging study aligned with this project and who had chosen to continue their participation through the additional phases of this project. Interested individuals were invited to contact the candidate by email for further information and to arrange a suitable time to conduct a phone screening. The initial phone screening determined whether the individual was eligible to participate in either

the control or SAD group, and to rule out exclusionary factors. Individuals who wished to proceed with their participation and were accepted into the study, received the participant information letter and booking information for available dates, the structure of participation and the process moving forward via email. Participants selected a suitable date combination for their participation and an appointment was made at their preferred time to attend in person at the ACU campus Fitzroy, Melbourne to complete their initial laboratory visit. The second laboratory visit was predetermined to occur on the provided day of the selected date combination at the standardised time of 2:30pm for all participants.

4.2. Participant Information, Screening and Clinical Assessment

4.2.1. General inclusion and exclusion criteria. The two participant groups were made up of equal numbers of male and female participants, including

a. *A clinical sample of social anxiety disorder participants and,*
b. *A sample of age and education matched healthy control participants*

Participants for both the healthy control and clinical SAD groups were required to be between the ages of 18 – 55 years of age, right handed and have no metal objects present in the body or which could not be removed (exclusion criteria for the larger MRI study was initially employed), be a non-smoker and have no substance abuse, be medication free, and own a smartphone (android or iOS). Healthy control participants were to have no current or suspected diagnosis of mental illness and not meet criteria for any mental illness (see screening procedures). The SAD participants were required to have a current or suspected diagnosis of SAD, with SAD criteria to be screened for (see screening procedures). Social anxiety disorder is commonly associated with comorbid disorders, such as generalised anxiety disorder (GAD) and depression (MDD) or low mood. Participants for the SAD group were included in the study if their principal diagnosis was SAD. Where there was the

presence of comorbid disorders, the following were to be accepted under the condition they were secondary to the diagnosis of SAD: GAD and symptoms of low mood (though not a current depressive episode). Participants with a comorbid or principal diagnosis of a current acute depressive episode, alcohol/substance abuse, bipolar disorder, schizophrenia, obsessive-compulsive disorder (OCD) and post-traumatic stress disorder (PTSD) were excluded. The presence of any other clinically significant medical (e.g. diabetes, cancer), neurodevelopmental disorder (e.g. Attention deficit / hyperactivity disorder) or neurological condition excluded individuals from participation. Participants were required to speak English fluently (e.g. English as a first language or 10 years or more speaking English and classifying themselves as fluent). Participants were required to be available between business hours and able to attend two lab visits at the candidate's university campus in Fitzroy Melbourne. Approximately two thirds of the way into testing, the exclusion criteria of right handedness and MRI safety (e.g. no metal objects in the body) were removed and recruitment was done outside of the MRI conditions in order to aid in recruitment for the SAD group. All participants provided written informed consent prior to their participation in the study.

4.2.2. Screening procedures. All participants underwent extensive screening to determine eligibility. Following completion of a general screening over the phone (see appendix A – 2), participants completed the Mini International Neuropsychiatric Interview Screen (MINI 7.0.2 Screen; English version for the DSM–5; Sheehan et al., 1998) to determine if any diagnostic modules of the full Mini International Neuropsychiatric Interview (MINI 7.0.2; English version for the DSM–5; Sheehan et al., 1998) were to be completed. Following the MINI 7.0.2 Screen, all SAD participants completed the corresponding Modules of the MINI 7.0.2 for any "Yes" responses (if required) and the full diagnostic Module F. Social Anxiety Disorder (Social Phobia) to assess if current DSM–5 diagnostic criteria for SAD was met. The following two measures were administered to all participants

according to the processes described above, assessing for the presence of neuropsychiatric disorders and to confirm the DSM–5 diagnostic criteria was met for all SAD participants.

- *Mini International Neuropsychiatric Interview Screen (MINI Screen) Version 7.0.2 for the DSM–5 (Sheehan et al., 1998).* The MINI Screen is a brief screening device which utilises only the screening questions for each of the 17 modules of the complete standard MINI 7.0.2. The screen determines that a "No" response to any of the screening questions for each disorder usually means it is unlikely the individual has the psychiatric disorder. A "Yes" response to any of the screening questions for each module in the standard MINI 7.0.2 prompts the clinician to ask additional questions using the full MINI 7.0.2 to determine if the diagnostic criteria for the psychiatric disorder is met. The MINI Screen was used for all participants to determine the presence of a psychiatric disorder and to prompt the clinician to utilise the complete standard MINI 7.0.2 modules where indicated.
- *Mini International Neuropsychiatric Interview (MINI) Version 7.0.2 for the DSM–5 (Sheehan et al., 1998)* is a comprehensive yet brief structured diagnostic interview for the major psychiatric disorders in the DSM- 5 and the International Classification of Disease (ICD – 10) criteria. The MINI is the most widely used psychiatric structured diagnostic interview instrument in the world. The MINI has shown to have similar reliability and validity to both the SCID (Structured Diagnostic Interview for the DSM; Spitzer, Williams, Gibbon, & First, 1992) and the CIDI (World Health Organisation Composite International Diagnostic Interview). The brevity in which the MINI may be administered (mean administration time of 15 minutes) made it the structured psychiatric interview of choice for this study. It can also be used by clinicians with only brief training. The standard MINI 7.0.2 assesses the 17 most common disorders in mental health, selected based on prevalence rates of 0.5% or higher in general population, with previous versions updated to map the DSM-5 diagnostic criteria (MINI Version 7.0.2). The MINI 7.0.2 was

used to assess the presence of 17 psychiatric disorders, and to assess for the inclusion criteria of SAD (as defined by the DSM-5) in the clinical sample.

4.2.3. Clinical assessment measures for further classification of SAD participants. The following measures were used to further characterise the symptom severity of social anxiety for all clinical SAD participants.

- *Social Interaction Anxiety Scale (SIAS; Mattick & Clarke, 1998)* is a self-report assessment used to assess social interaction anxiety, or an individual's tendency to avoid and fear social interactions due to fear of scrutiny and negative evaluation by others. Participants rated 20 items (e.g. I get nervous if I have to speak to someone in authority, like a teacher, boss, etc) using a 5-point Likert scale (0 = not at all characteristic or true of me; 4 = extremely characteristic or true of me) to indicate the degree to which they felt each statement was characteristic or true for themselves. Three of the items (item 5, 9, 11) are reverse scored, with all items then summed to provide a total raw score, with higher total scores representing greater social anxiety. Cut off scores of 36 are indicative of probable SAD (Peters, 2000), with average scores of individuals previously diagnosed with SAD 55.24 (n = 74; Peters, 2000). The SIAS exhibits high internal consistency reliability (Cronbach's $\alpha = 0.93$), high test-retest reliability and excellent discriminant validity. The SIAS was administered to all potential clinical SAD participants over the phone during the phone screening as an additional measure of SAD.
- *Liebowitz Social Anxiety Scale (LSAS; Liebowitz, 1987)* is a commonly used measure of social anxiety symptom severity designed to measure fear and avoidance experienced across a range of 24 social situations. The 24-item scale is divided into two subscales, 13 items pertaining to performance anxiety (e.g. performing in front of an audience) and 11 items concerning social anxiety (e.g. talking with people you don't know well). Items are first rated on a 4–point Likert scale (0 = none to 3 = severe) relating to fear or anxiety felt

during the situations, and then the same items are again rated on a 4-point Likert scale (0 = never to 3 = usually) for avoidance of the situations. Combining the total scores for both subscales gives a total raw score, with a maximum of 144. Severity of social anxiety is determined from this total raw score, with scores ranging between 30-50 being mild social anxiety, 50-65 moderate social anxiety, 65-80 marked social anxiety, 80-95 severe social anxiety and great than 90 pertaining to very severe social anxiety. The LSAS demonstrates good internal consistency, test-retest reliability, convergent and discriminant validity in SAD participants (Heimberg et al., 1999). The LSAS was used as an additional measure to confirm inclusion of SAD participants in the clinical group.

4.2.4. Other clinical assessment measures. The following commonly used standardised clinical measures were administered to assess state and trait anxiety, depression, self-esteem, fear of negative and positive evaluation, childhood maltreatment, attachment and personality as a part of the screening, testing and characterisation measures.

- *The State-Trait Anxiety Inventory* (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) is a self-report measure designed to assess state and trait anxiety. The most common version, Form-Y, contains 20 items assessing state anxiety (e.g. I am tense) and 20 items assessing trait anxiety (e.g. I am content). The 20 state items are rated to determine anxiety *at this moment* on a 4 point Likert scale (1 = not at all to 4 = very much so). Trait items are rated according to how the individually *generally feels* on a 4-point Likert scale (1 = almost never to 4 = almost always). Scores are summed to give a total raw score, with higher scores indicative of greater anxiety. The STAI demonstrates high internal consistency reliability (Cronbach's α = .86-.95), good test-retest reliability and good construct and concurrent validity.
- *The Depression Anxiety Stress Scale* (DASS-21; Lovibond & Lovibond, 1995) is designed to assess symptoms of depression, anxiety and stress. The 21-item scale is

divided into three 7-item subscales assessing depression (e.g. I felt that I had nothing to look forward to), anxiety (e.g. I felt scared without any good reason) and stress (e.g. I found it hard to wind down). Items are rated on a 4-point Likert scale (0 = did not apply to me at all to 3 = applied to me very much) of how much each statement applied over the past week. Scores for each subscale are calculated by summing the items for the relevant scale. The DASS-21 demonstrates adequate reliability for total scores (Cronbach's α = .93) and for the depression (α = .88), anxiety (α = .82), and stress (α = .90) scales (Henry & Crawford, 2005) and demonstrates adequate convergent and discriminant validity.

- *Rosenberg Self-Esteem Scale* (RSE; Rosenberg, 1965) is a measure designed to assess global self-worth through assessment of positive and negative feelings about oneself. The 10-item scale consists of statements dealing with general feelings about oneself (e.g. at times I think I am no good at all) rated on a 4-point Likert scale (1 = strongly disagree; 4 = strongly agree) of how much the individual agrees or disagrees with each statement. Scores lower than 15 are indicative of problematic low self-esteem. High internal consistency (α = .77) is demonstrated by the RSE (Rosenberg, 1965).
- *Brief Fear of Negative Evaluation Scale* (B-FNE; Leary, 1983) is a measure designed to assess anxiety associated with negative evaluation by others. The scale is comprised of 12 items describing fearful or worrying cognitions (e.g. I am frequently afraid of other people noticing my shortcomings). Participants rate each item using a 5-point Likert scale (1 = not at all characteristic of me; 5 = extremely characteristic of me). Higher total scores indicate increased anxiety associated with perceived negative evaluation by others. The B-FNE demonstrates high internal consistency (Cronbach's α = .90-.91).
- *Fear of Positive Evaluation Scale* (FPES; Weeks, Heimberg, & Rodebaugh, 2008) is a measure designed to assess fear of positive evaluation, a cognitive component of social anxiety. The measure comprises 10 statements pertaining to fear of positive evaluation

(e.g. It would make me anxious to receive a compliment from someone that I am attracted to). Each item is rated on a 10-point Likert scale of the degree to which the individual feels the statement is characteristic of themselves (0 = not at all true to 9 = very true). The measure demonstrates good internal consistency (Cronbach's α = .89), excellent test-retest reliability and good convergent and discriminant validity (Weeks et al., 2008; Weeks et al., 2012).

- *Childhood Trauma Questionnaire* (CTQ; Bernstein et al., 1994) is a screening tool to assess for histories of abuse and neglect. The 28-item self-report measure includes items pertaining to five types of maltreatment (e.g. I didn't have enough to eat), including emotional, physical, and sexual abuse, and emotional and physical neglect. Items are rated on a 5-point Likert Scale for how true a statement is according to when the individual was growing up (1 = never true to 5 = very often true). The measure has demonstrated very good evidence for reliability and validity among substance or alcohol dependent patients. In the substance and alcohol dependent patient population, high internal consistency scores (for sexual abuse, emotional neglect, emotional abuse, physical abuse, Cronbach's α = .93-.95, .88-92, .84-.89 and .81-.86, respectively) and test-retest reliability (calculated at close to 0.80) were demonstrated. Factor analysis on the five-factor CTQ model demonstrated good validity for structural invariance. Reliability and validity evidence for the CTQ among substance or alcohol dependent patients suggests the measure is suitable for the brief assessment of multiple dimensions of six types of childhood trauma (Fink, Bernstein, Handelsman, Foote, & Lovejoy, 1995).
- *Attachment Style Questionnaire* (ASQ; Feeney, Noller, & Hanrahan, 1994) is a self-report measure assessing adult attachment. The 40-item measure is comprised of statements (e.g. I prefer to keep to myself) pertaining to five factors, including confidence,

relationship as secondary, need for approval, discomfort with closeness and preoccupation with relationships. Items are rated on a 6-point Likert scale for how much the participant agrees with the statement (1 = totally disagree to 6 = totally agree) The ASQ demonstrates good reliability and validity in both clinical and nonclinical populations (Feeney et al., 1994; Ko, Hewitt, Cox, Flett, & Chen, 2019).

- *Big Five Inventory* (BFI; John & Srivastava, 1999) is designed to assess the Big Five factors (dimensions) of personality (i.e. openness, conscientiousness, extraversion, neuroticism, agreeableness). The self-report measure is comprised of 44-items describing a number of personality characteristics (e.g. I see myself as someone who is talkative). Items are rated on a 5-point Likert scale for how much the participant agrees with the statement (1 = disagree strongly to 5 = agree strongly). The BFI demonstrates good internal consistency reliability, factor structure, and convergent-discriminant validity, providing a succinct measure of the Big Five factors (John, Naumann, & Soto, 2008)

4.2.5. Subjective mood measures. A shortened 5–scale version of a visual analogue mood scale was administered at each sampling time point across the TSST, for a total nine assessments of subjective mood changes during lab 2.

- *Visual Analogue Mood Scale* (VAMS; Bond & Lader, 1974) measures mood across 16 scales, using horizontal 100m lines to represent the full range of each mood state. The brief VAMS designed for use in this thesis included Happy, Sad, Anxious, Tired and Withdrawn mood scales. Participants were asked to rate the way they felt at the time in terms of these five dimensions. The line represented the full range of each dimension (0 = a complete absence, 100 = the highest value of the mood). See Figure 4.1.

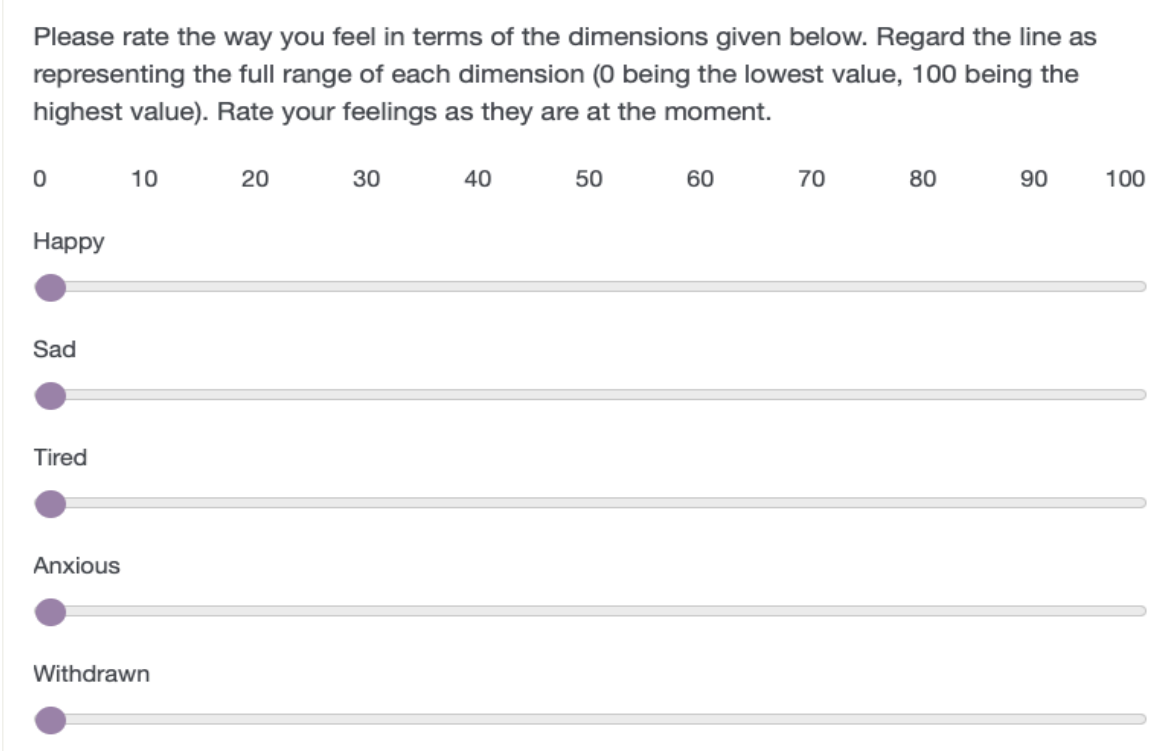


Figure 4.1. Brief VAMS designed to assess mood across acute psychosocial stress task lab

4.3. Study Design and Study Procedures

Individual study methodologies are detailed in each of the respective thesis chapters (Chapter 6 and 7). The following section will briefly detail the overall study protocol following participants recruitment, screening and inclusion in the study population.

4.3.1. General overview. Participation involved an 8-day testing cycle of 3 parts:

i. Lab-based sessions: Two sessions (1x60mins; 1x90mins) conducted in the laboratory at ACU (Melbourne Campus, Fitzroy). Completion of background and well-being questionnaires (Qualtrics blocks A and C completed in lab; block B completed at home) and participation set-up occurred in Lab 1, with the TSST completed in the second lab.

ii. Mobile App monitoring: Using the EMA hosting application SEMA2 (Harrison, Harrsion, Koval, Gleeson, & Alvarez, 2017) on participants own smartphone devices

(Android/iOS), feelings and behaviour were monitored via a series of prompts involving answering a brief questionnaire multiple times per day over 7 days (~15mins per day).

iii. At-home Saliva collection: During mobile app monitoring, participants also completed 3 days of saliva sampling, including both across the day salivary cortisol and the cortisol awakening response (CAR; first hour post awakening). See Figure 4.2 and Table 4.1.

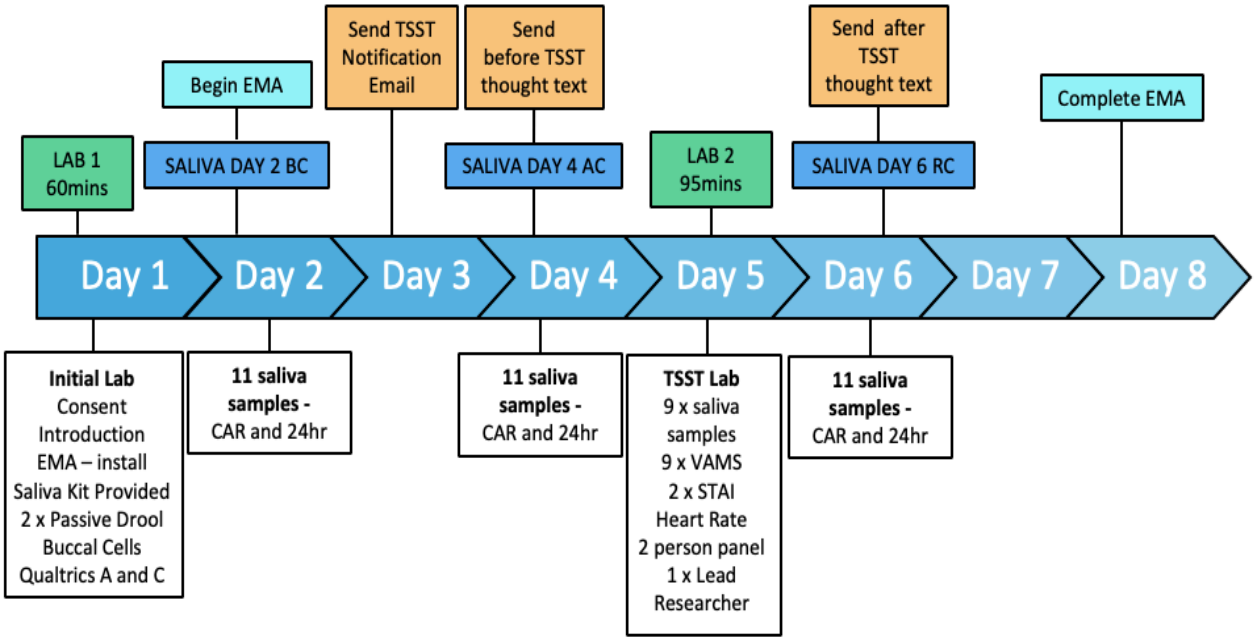


Figure 4.2. Overview of protocol

Scheduling of participants – general. The nature of the protocol design and structure meant Lab 2 must occur a minimum of 5-days post Lab 1 (i.e. on Day 5, with Lab 1 on Day 1). The lab visits followed a set sequence to accommodate the desired baseline, anticipatory and recovery phases of testing. There was no room for participants to reschedule Lab 2 once commencing the testing cycle. If a participant was unable to complete the full 8-day cycle in one sequence, they were excluded (even if they had commenced their testing).

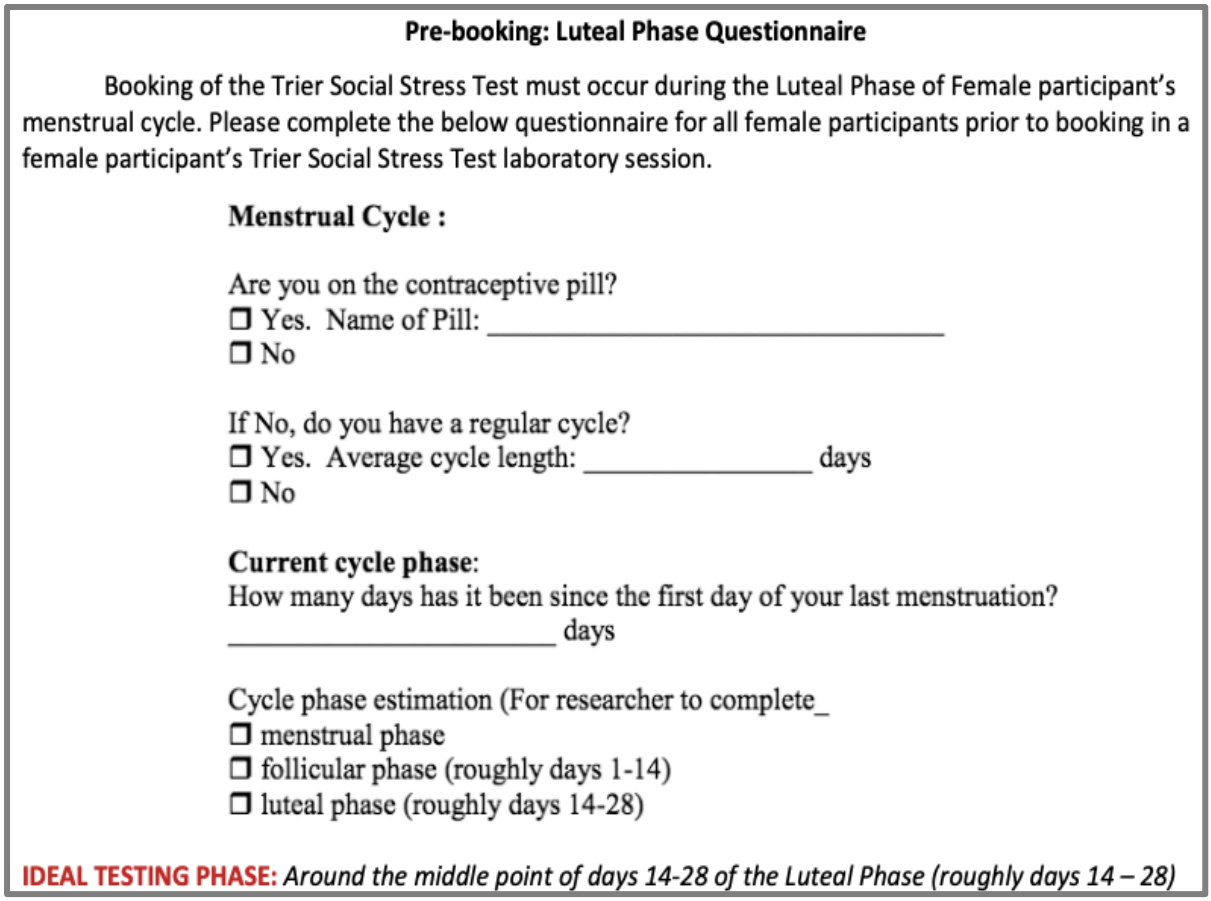
Table 4.1

Description of Protocol

	Description	Protocol completed	Saliva collection
Day 1	Initial Lab Session – 60mins	• Consent • Qualtrics Part A and C completion (background/demographics and clinical measures) – approximately 40 mins; self – report • Buccal Cell Collection/ Baseline Saliva 2 x 1ml – passive drool • EMA Introduction – Practice/install of app • Participant Saliva Kit – instructions for	Nil
Eve – Day 1	Evening of Day 1	• Qualtrics Part B sent to Participant via email link to complete at home post initial lab – approximately 30 mins; self – report	Nil
Day 2	EMA – Day 1 (Full)	• First full day of EMA completed with saliva – unaware of TSST	**BASELINE Cortisol** – 24hr collection and CAR Sampling; total 11 samples
Day 3	EMA – Day 2 (Full)	• Second full day of EMA completed without saliva – unaware of TSST • **TSST notification email sent in evening**	Nil
Day 4	EMA – Day 3 (Full)	• Third full day of EMA completed with saliva – awareness of upcoming TSST • **PRE-TSST "thought text" sent in evening**	**ANTICIPATORY Cortisol** – 24hr collection and CAR Sampling; total 11 samples
Day 5	Lab 2 TSST Session – 90mins	• EMA completed as per usual + TSST Laboratory Session at 2:30pm in the afternoon	9 x TSST Cortisol samples
Day 6	EMA – Post TSST 1	• First full day of Post – TSST EMA completed with saliva • **POST-TSST "thought text" sent**	**RECOVERY Cortisol** – 24hr collection and CAR Sampling; total 11 samples
Day 7	EMA – Post TSST 2	• Second full day of Post – TSST EMA completed without saliva • **Completed saliva sampling kit returned via express post satchel provided during Lab 1**	Nil
Day 8	EMA – Post TSST 3	• Third and final full day of Post – TSST EMA completed without saliva	Nil

Note. The testing cycle was standardised for all participants. Study phases were completed following the same timeline and Lab 2 followed a strict standardised TSST protocol.

Scheduling of participants – females. Given the fluctuations in sex hormones during the menstrual cycle, female participants completed the TSST (Lab 2) within the luteal phase of their menstrual cycle (i.e. between 14 to 28 days since the first day of the start of their last period of a regular 28-day cycle; see Study 1 for justification). During screening, female participants' luteal phase was determined (see Figure below for screening of menstrual cycle phases) and only Lab 2 dates available within the individual's luteal phase were offered for selection. Should the female individuals cycle begin early or fall outside of the scheduled date for Lab 2, the participant was rescheduled to the following month (i.e. cycle).



Pre-booking: Luteal Phase Questionnaire

Booking of the Trier Social Stress Test must occur during the Luteal Phase of Female participant's menstrual cycle. Please complete the below questionnaire for all female participants prior to booking in a female participant's Trier Social Stress Test laboratory session.

Menstrual Cycle :

Are you on the contraceptive pill?
❒ Yes. Name of Pill: _____
❒ No

If No, do you have a regular cycle?
❒ Yes. Average cycle length: _____ days
❒ No

Current cycle phase:
How many days has it been since the first day of your last menstruation?
_____ days

Cycle phase estimation (For researcher to complete_
❒ menstrual phase
❒ follicular phase (roughly days 1-14)
❒ luteal phase (roughly days 14-28)

IDEAL TESTING PHASE: *Around the middle point of days 14-28 of the Luteal Phase (roughly days 14 – 28)*

Figure 4.3. Luteal phase questionnaire to determine Luteal Phase prior to scheduling.

4.3.2. At home saliva sampling. The response to stress occurs through two primary adaptive stress systems, the autonomic nervous system and hypothalamic pituitary adrenal (HPA) axis (Boyle, 2013; Compas, 2006). Salivary cortisol is widely known to reflect HPA

axis functioning in response to stress. Healthy HPA axis functioning is characterised by a strong diurnal cortisol rhythm, with deviations from expected normal diurnal cortisol linked to disease processes (Chrousos, 2009; Hoyt, Ehrlich, Cham, & Adam, 2016; Nader, Chrousos, & Kino, 2010). Three key components are typical of the diurnal cortisol rhythm: the diurnal cortisol slope, the area under the curve and the cortisol awakening response (CAR; a marked increase in cortisol secretion observed in the first hour post-awakening). Existing evidence demonstrates a steeper decline in cortisol slope is associated with improved health and psychosocial functioning (Adam, 2006; Hoyt et al., 2016; Huppert, 2006), while a flattened slope has been linked to chronic stress exposure (Adam & Gunnar, 2001; Miller, Chen, & Zhou, 2007) and disease processes (Abercrombie et al., 2004; Heim, Ehlert, & Hellhammer, 2000; Matthews, Schwartz, Cohen, & Seeman, 2006). Associations between daily average cortisol and stress or other health variables are inconsistent (Hoyt et al., 2016). Very large or very small average daily cortisol (i.e. area under the curve) is representative of poorer psychological and physiological functioning (Saxbe, 2008). The CAR has been shown to be altered in psychopathology (Chida & Steptoe, 2009). Higher observed CAR predicts future development of major depressive disorder (Vrshek-Schallhorn et al., 2013) and initial onset of anxiety disorders (Adam et al., 2014). Individuals with long-term anxiety disorders, specifically generalised anxiety disorder (GAD), have shown reduced or attenuated CAR (Hek et al., 2013) which has been attributed to chronically altered cortisol concentrations due to chronic overloading of the stress response (Berger et al., 2017; Saxbe, 2008).The HPA axis has been shown to be highly socially-sensitive (Dickerson & Kemeny, 2004), with the CAR particularly shown to be responsive to social challenges an individual may face in daily life. Increased negative subjective experience has been associated with higher CAR AUCi the following day (Adam et al., 2014).

In order to assess the diurnal cortisol rhythm across the testing cycle, participants completed 3 full days of at-home saliva sampling, which included sampling of across the day salivary cortisol (e.g. between 8am and 10pm) and the CAR in the first hour post awakening (4 samples at 0, 30, 45 and 60 min post awakening). The at home salivary cortisol sampling equated to 11 samples per day (4 CAR; 7 across the day) for a total of 33 samples per participant (see Figure 4.4). The three days were in accordance with the specific design phases of the protocol – baseline cortisol (Day 2; e.g. no awareness of TSST); anticipatory cortisol (Day 4; e.g. awareness of TSST); recovery cortisol (Day 6; e.g. post- TSST).

Figure 4.4. Images of the at – home saliva collection kit provided to participants

For convenience, minimal participant burden and the benefit of no specialised training required, samples were collected by absorbing saliva onto an oral swab (i.e. SOS; SalivaBio

Oral swabs from Salimetrics). As cortisol was the only analyte of interest for this study, the SOS swabs were a suitable choice for ease of use for multiple collections and for analysis of cortisol only. The collection of saliva and the saliva kit protocol was explained in detail to participants during the initial introductory lab, along with all participants being provided a saliva collection information and record book (see Appendix A – 5). Participants were instructed to be as accurate as possible and to carefully track the specific details of their sampling. Where deviations from the desired sampling protocol occurred, participants were encouraged to record down the details in the notes section for the researchers to consider. Participants were provided a saliva kit, clearly labelled for each of the collection days – with each saliva vial individually labelled (see Figure 4.4). Upon completion of salivary cortisol sampling, participants were instructed to return their collection kit and record booklet via a pre-paid express post satchel provided during the introductory lab session.

4.3.3. Ecological momentary assessment – via the hosting application SEMA2. During the initial lab, participants completed an introductory lesson to SEMA (Harrison et al., 2017). Participants downloaded the SEMA application from the Apple App store or Android Google Play onto their smartphone device and logged into the application using a pre-determined SEMA ID and password provided by the Lead Researcher (see Figure 4.5). For the EMA component of the protocol, the SEMA app prompted participants on their own smartphone device with a survey to be completed 10 times per day. Surveys were sent via fixed intervals of 72 MINS±20 MINS between 9am to 9pm. Participants were informed that each survey was time-stamped, with a 15-minute expiry in order to minimise retrospective recall bias. They were informed that they should complete each survey as close to the initial notification as was safely possible. Any survey not completed and submitted wholly before the 15-minute expiry would disappear and count as a missed survey.

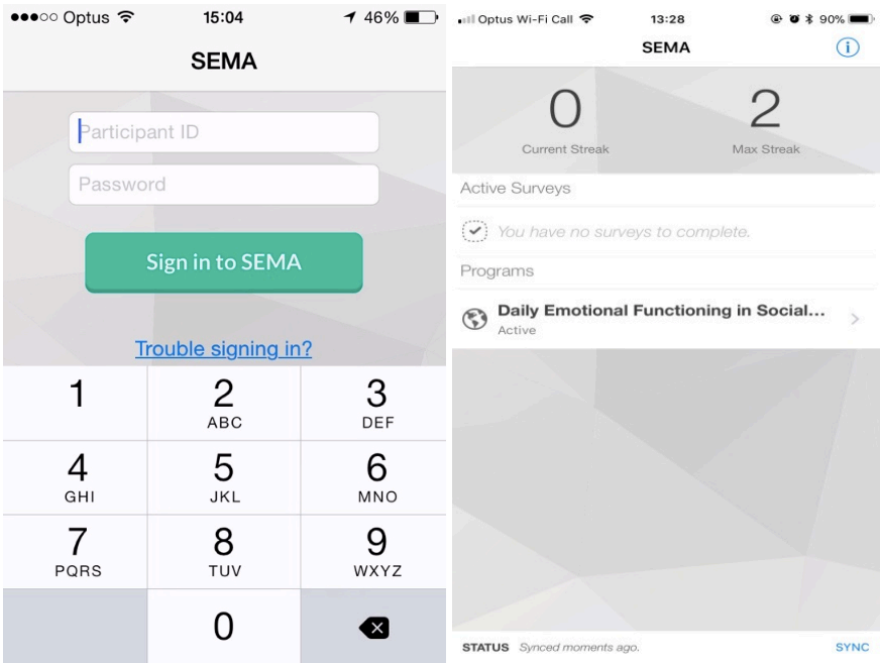


Figure 4.5. SEMA application user sign in and user interface.

Participant reimbursement. To maximise survey compliance and encourage survey completion, participant compensation for the entire study protocol totalled to a potential $150.00, with a minimum amount of $130.00. The financial compensation was calculated on a pro-rata basis dependent upon the participants survey compliance (e.g. response rate percentages of completed surveys; structured in a similar manner to previous studies, see Kashdan & Farmer, 2014). The maximum payment was provided if participants held a completed survey compliance between 70-80%, with compliance rates < 70% incurring the $20.00 deduction in final payment. Payment was provided in Coles/Myers vouchers.

4.3.4. Ecological momentary assessment measures. Items included in the ambulatory assessment component of the protocol measured momentary affect, state self-esteem, occurrence of events, threat awareness and environment context.

Affect. Five questions captured momentary affect. Including four basic emotions (both positive, e.g. *Right now*, how happy do you feel? and negative, e.g. *Right now*, how sad/anxious/angry do you feel?) and one self-conscious emotion (e.g. *Right now*, how

embarrassed do you feel?). Responses were rated on a sliding scale of 0 (not at all) to 100 (extremely).

State self-esteem. Three questions captured state self-esteem. These items were derived from the Heatherton and Polivy (1991) State Self-Esteem Scale (SSES). Heatherton and Polivy (1991) determined three factors for state self-esteem: performance, appearance and social state self-esteem. The highest loading item on each of these three factors was used to capture performance, appearance and social state self-esteem in this study. These included the appearance based self-esteem item (e.g. *Right now*, I am pleased with my appearance), the performance based self-esteem item (e.g. *Right now*, I feel confident about my abilities) and the social based self-esteem item (e.g. *Right now*, I am worried what other people think of me). Response options were rated on a sliding scale of 0 (not at all) to 100 (extremely).

Occurrence of events. Two primary questions captured the occurrence of events since the last survey. These were divided into positive or pleasant events (e.g. *Since the last survey*, has a positive/pleasant event occurred?) and negative or unpleasant events (e.g. *Since the last survey*, has a negative/unpleasant event occurred?). Response options were a simple Yes or No. The two occurrence of event questions were then succeeded by two branching questions, determined by a Yes or No occurrence of event response. For any Yes response to the occurrence of an event (Positive or Negative), two context questions were then displayed, these established whether the event was social in context (e.g. Did the event involve the possibility of being judged or scrutinised by others e.g. a social event?; Yes/No Response) and the interpretation of the event (e.g. Was the event threatening or non-threatening?; threatening/non-threatening response). For any No response to the occurrence of an event (Positive or Negative), two follow up questions were then displayed, these established whether there was the presence of avoidance (e.g. Have you actively avoided a situation that

could involve being judged by others?; Yes/No response) or rumination (e.g. Have you been preoccupied thinking about and earlier Negative/Unpleasant Event?; Yes/No response).

Attention toward threat. One question captured threat reactivity regarding attention to threat in the environment (e.g. *Since the last survey*, I have noticed people judging or scrutinising me). The response was rated on a sliding scale (0 = not at all to 100 = extremely).

Environment context. Three questions captured environmental context. One question captured who time was spent with since the last survey (e.g. *Since the last survey*, how have you spent most of your time?) Responses included six options (alone; with family members you're close with; with family members you're not close with; with close friends; with familiar company e.g. co-workers/acquaintances - not family; with strangers). One question captured the environment in which time was spent since the last survey (e.g. *Since the last survey*, in which environment have you spent most of your time?) with four response options (at Home; in a Private Space - not at home; in a Public Space – with others present); in a Public Space – with no others present). One question captured environment security (e.g. How secure have you felt in the environment you have spent most of your time?). Responses were rated on a sliding scale (0 = not at all to 100 = extremely).

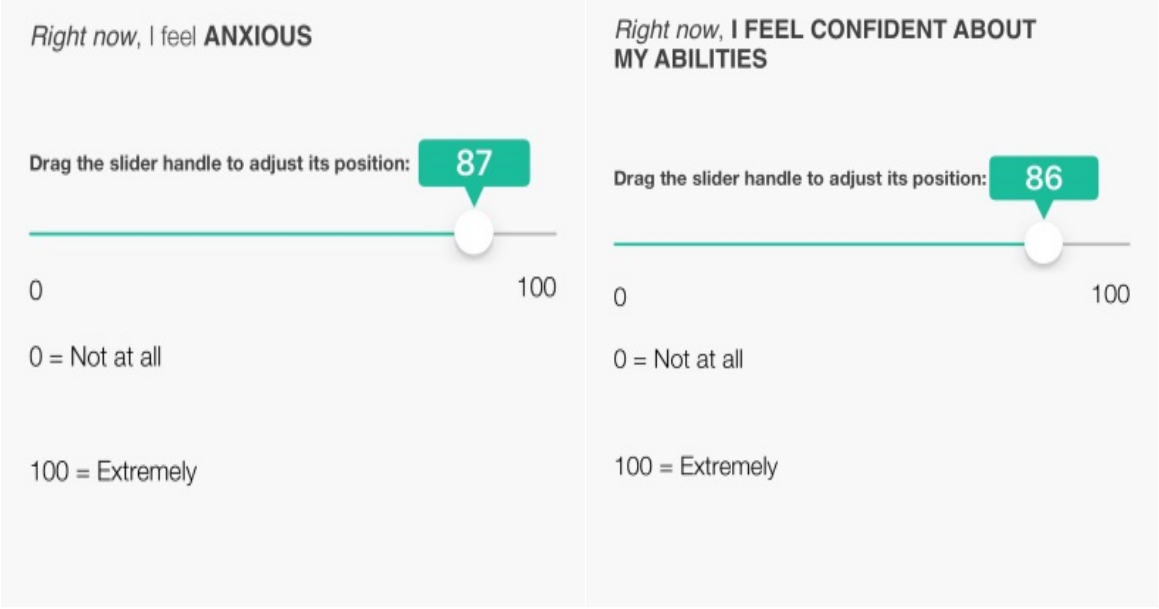


Figure 4.6. A selection of images of the SEMA2 application demonstration survey

Participant compliance . The 12th version of the *Daily Emotional Functioning in Social Anxiety Disorder* survey was used for all enrolled participants. 105 participants provided a total of 6999 survey iterations. Of these, nine pilot data sets were removed (i.e. colleagues who piloted the final version of the SEMA survey). Nine further empty data sets (i.e. no surveys answered) were removed for participants who had enrolled in SEMA but did not commence participation due to cancellation, loss of contact or change of mind. A total of 87 data sets formed the raw SEMA data file. Six final participant data sets were removed, two participants did not complete the full protocol (i.e. no TSST), one participant dropped out due to illness, one participant dropped out due to other commitments, one participant withdrew prior to commencing participation, and a final participant was cancelled early due to low compliance and problematic participation. A final 81 data sets were then run through the *cleansema* program (Murphy, 2017) in RStudio (RStudio Team, 2015). *Cleansema* is developed for initial data cleaning of surveys collected through SEMA, performing a number of functions on the raw data file, including: coding of new variables to determine survey compliance (e.g. has_answers, surveys_received/surveys_responded); removal of fast response times (response times < 500ms removed), and calculation of various date and time variables to assist in analyses (e.g. weekend/weekday). After cleaning, 6114 iterations were included in the final analyses. The mean number of surveys received was 75.48 (*SD* = 6.29; range 60-95) with participants completing an average 79.35% (*SD* = 11.42%; range = 51-100%) of all scheduled surveys.

4.3.5. Acute psychosocial stressor – The Trier Social Stress Test. During Lab 2 on Day 5, participants completed the TSST (Kirschbaum et al., 1993) – a reliable biopsychological tool commonly used and considered to be the gold standard for inducing an acute stress response in humans. The tool was utilised in order to explore the effects of acute stress on subjective and physiological stress functioning. For a detailed methodology of the

TSST protocol, refer to Chapter 5 of this thesis, which includes the publication *An introductory guide to conducting the Trier Social Stress Test* and the supplementary manual, which provides a detailed step-by-step guide of how to conduct the TSST.

4.3.6. Communication templates – TSST notification email and thought texts.

This thesis protocol included communication templates for use with participants. Participants received a TSST notification email in the evening of Day 3 (sent at a standardised time of 7pm, with confirmation of receipt required to be sent by participants), a thought text in the evening of Day 4 prior to Lab 2 and the evening of Day 6 post Lab 2. The TSST notification email informed the participant they would be completing a social task during Lab 2 and to set the boundary for the anticipatory phase of this study (phases included baseline; anticipatory and recovery). The two text messages were used to provide a measure of how much the participant had thought about the social task pre- and post-completion of Lab 2. Participants were required to respond to the two text messages prior to the end of the evening they were received on (standardised to be sent at 7pm). For communication templates, see below.

i. ***Email:*** Dear ____, I hope you are having a nice week and the participation is all going well so far. Your SEMA survey compliance is at __%. I really appreciate your effort.

   As you were notified during your Lab 1, here is your ___ evening email updating you on your second lab visit this coming ___ .

   For your second visit this ___ afternoon you will be ***completing a brief social task*** that will replicate a social experience you may encounter in your every day to day life, such as a first date. I ask that you Meet me at the level 3 lifts of the Mary Glowrey Building at around 2:30pm this coming ___ (or as close to this time as possible).

   The Lab Visit will take around 80 minutes (Departure time is shortly after 4pm).

A reminder also that tomorrow is your second day of saliva collection, of both your CAR response for the first hour after awakening and the 7 other samples at the set timepoints across the day.

Please confirm you have received and read this email before turning in for the evening, and further, let me know if you have any queries or concerns prior to your Lab 2. Otherwise, I will see you ____ afternoon at 2:30pm.

ii. ***Thought text – before*:** Hi _____, I hope you're having a nice evening.

As a part of your participation we have one quick question for you this evening - can you please text me your response to the following question:

On a scale of 1 (not at all) to 10 (all the time), how much have you thought about tomorrow's social task?

iii. ***Thought text – after.*** Hi _____, I hope you're having a nice evening.

As a part of your participation we have one quick question for you this evening - can you please text me your response to the following question:

On a scale of 1 (not at all) to 10 (all the time), how much have you thought about yesterday's social task?

4.4. General Data and Statistical Analysis.

Specific details of statistical analyses and software for the two empirical studies are detailed within their respective chapters (Chapter 6 and 7). Broadly, four data analysis packages were utilised in this thesis: Statistical Package for the Social Sciences (SPSS; IBM Corp, 2015), Stata (StataCorp, 2019), R (R Core Team, 2014) using the interface RStudio (RStudio Team, 2015) and JASP (JASP Team, 2019). These statistical packages are widely used. SPSS and Stata are commercially available, with R and JASP accessible free of charge.

4.5. Population Demographics and Clinical Characterisation

Table 4.2

Group Demographic Characteristics and Endogenous Hormones of Social Anxiety Disorder (SAD) and Healthy Control (HC) Participants

N = 81		SAD (*n* = 40)	HC (*n* = 41)	Test	*p*	*df*
Gender (Female)	*n (%)*	20 (50.0)	20 (48.8)	$\chi^2 = 0.01$	.913	1
Age (Years)	*M (SD)*	28.42 (7.90)	25.75 (6.36)	$t = 1.68$	.098	79
Education (Total Years)	*M (SD)*	16.18 (2.25)	16.57 (2.09)	$t = 0.83$	.412	79
Education (Level Completed)				*FE*	.617	1
Primary & Secondary School	*n (%)*	9 (22.5)	14 (34.1)			
TAFE, Certificate, Diploma	*n (%)*	5 (12.5)	3 (7.3)			
Tertiary Degree	*n (%)*	21 (52.5)	18 (43.9)			
Postgraduate Tertiary Degree	*n (%)*	5 (12.5)	6 (14.6)			
Hormonal Contraceptives	*n (%)*	7 (17.5)	12 (29.3)	$\chi^2 = 1.56$	.211	1
Contraceptive Pill	*n (%)*	4 (10.0)	11 (26.8)			
Inter-Uterine-Device/Implant	*n (%)*	3 (7.5)	1 (2.4)			
None	*n (%)*	13 (32.5)	8 (19.5)			
Menopause	n (%)	0 (0)	0 (0)			
Medication	n (%)	0 (0)	0 (0)			
Smoking During Participation	n (%)	0 (0)	0 (0)			
SAD Screening and Severity						
MINI SAD Criteria Met	n (%)	40 (100)	0 (0)			
SIAS	*M (SD)*	57.00 (9.26)	16.08 (10.30)	$t = 18.69$	< .001***	78
LSAS	*M (SD)*	78.70 (19.05)	24.26 (16.25)	$t = 13.21$	< .001***	73
Endogenous Hormones		SAD (*n* = 46)	HC (*n* = 39)			
Gender (female)	*n (%)*	24 (52.17)	22 (56.41)			
Oxytocin (pg/ml Saliva)	*M (SD)*	1.43 (0.33)	1.47 (0.41)	$t = 0.46$	.171	83
Vasopressin (pg/ml Saliva)	*M (SD)*	1.75 (0.46)	1.87 (0.55)	$t = 1.12$	.083	83

Note: MINI = MINI international neuropsychiatric interview version 7.0.2, SIAS = social interaction anxiety scale, LSAS = Leibowitz social anxiety scale, *M(SD)* = mean (standard deviation), *n* (%) = number (percentage), *df* = degrees of freedom, SAD = social anxiety disorder, HC = healthy control, χ^2 = chi-square test, *t* = t -test of independence, *p* = *p*-value significant at $p < 0.05$ level, * $p < .05$. ** $p < .01$. ***$p < .001$

Table 4.3

Group Clinical Characteristics for Social Anxiety Disorder (SAD) and Healthy Control (HC) Participants

N = 81		SAD (*n* = 40)		HC (*n* = 41)		Test	*p*	*df*
Clinical Characteristics								
STAI (Trait)	*M (SD)*	55.10	(7.58)	36.05	(6.93)	*t* = 11.67	< .001***	77
DASS-21 Depression	*M (SD)*	17.00	(8.61)	3.56	(3.62)	*t* = 9.20	< .001***	79
DASS-21 Anxiety	*M (SD)*	18.85	(8.18)	4.15	(5.34)	*t* = 9.61	< .001***	79
DASS-21 Stress	*M (SD)*	24.70	(8.16)	8.29	(5.96)	*t* = 10.35	< .001***	79
RSE	*M (SD)*	14.15	(4.32)	22.49	(4.34)	*t* = 8.67	< .001***	79
FPES	*M (SD)*	41.65	(13.41)	20.70	(11.83)	*t* = 7.46	< .001***	79
BFNE	*M (SD)*	49.90	(5.53)	32.46	(8.78)	*t* = 10.67	< .001***	79
Attachment - ASQ								
Confidence	*M (SD)*	25.58	(6.36)	35.46	(5.41)	*t* = 7.35	< .001***	75
Relationship as Secondary	*M (SD)*	20.32	(5.13)	16.03	(5.11)	*t* = 3.65	< .001***	74
Need for Approval	*M (SD)*	30.05	(4.22)	20.74	(6.15)	*t* = 7.72	< .001***	75
Discomfort with Closeness	*M (SD)*	43.00	(7.69)	32.13	(7.87)	*t* = 6.17	< .001***	76
Relationship Preoccupation	*M (SD)*	33.67	(6.66)	24.28	(6.77)	*t* = 6.17	< .001***	76
Personality - BFI								
Openness to Experiences	*M (SD)*	3.82	(0.58)	3.63	(0.62)	*t* = 1.37	.174	78
Conscientiousness	*M (SD)*	3.22	(0.60)	3.76	(0.66)	*t* = 3.83	< .001***	78
Extraversion	*M (SD)*	2.35	(0.66)	3.38	(0.72)	*t* = 6.68	< .001***	78
Agreeableness	*M (SD)*	3.48	(0.65)	3.88	(0.55)	*t* = 2.92	.005**	78
Neuroticism	*M (SD)*	3.94	(0.59)	2.61	(0.72)	*t* = 9.00	< .001***	78
Environmental - CTQ								
Emotional Abuse						*FE*	.002**	
None to Minimal	*n (%)*	17	(45.95)	33	(82.50)			
Low to Extreme	*n (%)*	20	(54.05)	7	(17.50)			
Physical Abuse						*FE*	.026*	
None to Minimal	*n (%)*	22	(57.90)	36	(87.80)			
Low to Extreme	*n (%)*	16	(42.10)	5	(12.20)			
Sexual Abuse						*FE*	.044*	
None to Minimal	*n (%)*	29	(74.35)	37	(92.50)			
Low to Extreme	*n (%)*	10	(25.65)	3	(7.50)			
Physical Neglect						*FE*	.181	
None to Minimal	*n (%)*	21	(53.85)	30	(73.17)			
Low to Extreme	*n (%)*	18	(46.15)	11	(26.83)			
Emotional Neglect						*FE*	< .001***	
None to Minimal	*n (%)*	11	(28.20)	30	(73.17)			
Low to Extreme	*n (%)*	28	(71.80)	11	(26.83)			
Minimisation Denial						*FE*	.043*	
None to Minimal	*n (%)*	33	(82.50)	26	(63.41)			
Low to Extreme	*n (%)*	7	(17.50)	15	(36.59)			

Notes: M(SD) = mean (standard deviation), *n (%)* = number (percentage), *df* = degrees of freedom, SAD = social anxiety disorder, HC = healthy control, SIAS = social interaction anxiety scale, LSAS = Leibowitz social anxiety scale, STAI = state trait anxiety inventory, DASS-21 = depression anxiety stress scales, RSE = Rosenberg self-esteem scale, FPES = fear of positive evaluation scale, BFNE = brief fear of negative evaluation scale, ASQ = attachment style questionnaire, BFI = big five inventory, CTQ = childhood trauma questionnaire, *FE* = Fisher's exact test of independence, *p* = *p*-value significant at $p < 0.05$. * $p < .05$. ** $p < .01$. *** $p < .001$

CHAPTER 5. Publication – An Introductory Guide to Conducting the Trier Social Stress Test

5.1 Preamble

This chapter presents the final accepted version of a peer-reviewed and published manuscript. The manuscript is unaltered from the published article. Directly following the manuscript is the supplementary manual, providing a step-by-step guide for conducting the Trier Social Stress Test.

Citation:

Grace, C., Labuschagne, I., Rendell, P., Terrett, G., & Heinrichs, M. (2019). An introductory guide to conducting the Trier Social Stress Test. *Neuroscience and Biobehavioral Reviews*, *107*, 686-695. doi: https://doi.org/10.1016/j.neubiorev.2019.09.032

5.2 Statement of Contribution

In the case of Chapter 5, the nature of my contribution to this manuscript was the conception, planning, intellectual input, drafting and revising of the article. The extent of my contribution was 65%. Authorship was shared with Dr Izelle Labuschagne at 25%, who contributed significantly to the conception, planning, intellectual input, drafting, submission and revising of the article. Professor Markus Heinrichs contributed 5% and Professor Peter Rendell and Assoc. Prof Gill Terrett equally contributed the remaining 5%, all providing intellectual input and comments on the manuscript.

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5.3 Highlights

- The Trier Social Stress Test (TSST) is a robust tool to induce acute psychobiological stress.
- Yet, clear methodological guidelines for conducting the TSST are not available.
- We provide an accessible step-by-step introductory guide for conducting the TSST.
- These guidelines may be modified depending on to the individual research question.
- We hope to provide a valuable resource for researchers and improve the scientific standard in this field of research.

5.4 Abstract

The Trier Social Stress Test (TSST) is a reliable biopsychological tool to examine the effects of acute stress on psychological and physiological functioning in humans. While the TSST reliably increases hypothalamic-pituitary-adrenal axis activation, amongst other biomarkers, through a combination of social evaluative threat and uncontrollability, the original protocol is limited in methodological detail that has impacted its reproducibility. Although many studies include a mock job interview and surprise arithmetic task, there are large variations in the timing of events, the number and method of biological (e.g., cortisol) sampling, the administration of a glucose drink, set-up of equipment and rooms, panel composition, and panel interaction with participants. We provide an overview of the potential impact of methodological variations on the stress (cortisol) response. Importantly, we also provide a step-by-step guide as a laboratory manual on how to conduct the TSST. This introductory guide may be a useful and time-saving resource that may also improve the scientific standard and reliability of the reported psychobiological stress effects in future studies.

Keywords: cortisol, HPA axis, humans, public speaking, mental arithmetic, TSST

5.5. MANUSCRIPT

1. Introduction

Emotional, physical and environmental stressors permeate several domains of daily living. For decades, stress has been researched in many experimental laboratories (Maes et al., 1998; Mason, 1975) and a wealth of knowledge about the impact of stress on human functions has been gained. Yet, stress is a complex function that affects various psychological and biological systems, particularly the hypothalamic-pituitary-adrenal (HPA) axis. Over time, chronic stress can have severe negative consequences on the population's health and well-being by contributing to the manifestation of many psychosomatic and psychiatric illnesses. Not surprisingly, stress has been reported by the World Health Organization as one of the most significant health concerns of the 21st century (Bebbington, 2001). Therefore, importance of studying stress, especially regarding the association between stress and various physical and mental health problems, is abundantly clear.

The present paper aims to describe the main methodological details and to provide a step-by-step introductory guide for the administration of the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993), a commonly used and very reliable laboratory tool for the induction of a strong psychobiological stress response in humans. The novel contribution of this paper is the step-by-step introductory guide presented in the **Supplementary material** that provides much-needed information on how to conduct the TSST given that there are very limited details on the exact methodology of the TSST from past studies. This paper and guide are meant to be accessible for the applied researchers in psychology, neuroscience, psychiatry and allied health. Given the broad application of the TSST across fields, we further highlight that this is only an introductory guide that may be most useful to those starting out with the TSST for the first time and can be adapted based on specific research questions. We also highlight that our goal with this paper was not to provide a comprehensive review of the literature and its limitations, as this can be found elsewhere (e.g. Allen, Kennedy, Cryan, Dinan, & Clarke, 2014; Allen et al., 2017; Birkett, 2011; Goodman, Janson, & Wolf, 2017; Kudielka et al., 2007).

The first section of the paper introduces the psychological, physiological and neurohormonal mechanisms involved in the human stress response. This is important for a better appreciation of the complexity of the stress response and factors that could influence this response. The second section of the paper provides a brief description of the TSST. This includes a discussion of the implications of variations to key methodological aspects of the administration of the TSST as evident from past studies.

2. The Neurobiology of Stress

The body's response to stress serves a protective purpose, as it prepares the body to combat perceived or actual disruptions to homeostasis, or minimise the impact on the organism through activation of the fight, flight or freeze response (Ulrich-Lai & Herman, 2009). Several complex interconnected physiological systems are activated in response to stress, with these forming the basis for the adaptive survival mechanisms of the human body in the face of threat (Compas, 2006). Mechanistically, the two primary adaptive stress response systems consist of the autonomic nervous system, which involves the sympathetic and parasympathetic branches of the peripheral nervous system, and the HPA axis that integrates both the central nervous and endocrine systems (Compas, 2006).

As part of the autonomic nervous system via its *sympathetic* branch, the sympathetic adrenal medullary system, provokes the most immediate "fast" response to stress through activation of the adrenal medulla and release of epinephrine and norepinephrine into the blood that causes rapid changes in physiological states (e.g. increased heart rate and blood flow due to excitation of the cardiovascular system; Ulrich-Lai & Herman, 2009). This response is also sometimes referred to as our "fight and flight" response. This autonomic nervous system excitation is brief due to the reflex activation of the *parasympathetic* branch, which acts to restore functions and relax the body by slowing and maintaining the body's basic needs (Boyle, 2013; Murison, 2016). By comparison, activation of the HPA axis is a relatively "slow" response, and involves the hypothalamus acting on the anterior pituitary gland to release adrenocorticotropic hormone into systemic circulation to reach its primary target

organ, the adrenal cortex. Peak levels of plasma glucocorticoids (e.g., cortisol) are then released approximately ten minutes post initiation of a stressor and act to maintain homeostasis in the body (Droste et al., 2008).

However, there are various additional human systems involved in the stress response, including the immune system, the enteric (gastrointestinal) nervous system, other endocrine systems such as the thyroid and somatotropic axes, cognitive functions, such a reappraisal of the stressor, and even the brain's structure and function (De Kloet, Joëls, & Holsboer, 2005; Murison, 2016; Steptoe, Hamer, & Chida, 2007; Ziegler, 2012). Additionally, the hormones involved in the stress response are also more widespread than those mentioned here; for a review see Allen et al. (2014).

While these adaptive stress response systems are highly functional, long-term exposure to stress can lead to debilitating consequences. Chronic stress significantly increases an individual's vulnerability to detrimental medical outcomes, including autoimmune diseases, cardiovascular diseases, endocrine disorders, and obesity (Chrousos, 2009; Dallman et al., 2003; Khanam, 2017; Miller, Chen, & Zhou, 2007; Steptoe & Kivimaki, 2012; Stojanovich & Marisavljevich, 2008). In addition, stress is associated with increased vulnerability to a number of mental health conditions, with evidence showing it to be an important predictor of anxiety and depression (D'Angelo & Wierzbicki, 2003; Parrish, Cohen, & Laurenceau, 2011). Furthermore, individuals faced with chronic stress have an increased likelihood of engaging in substance abuse and are more vulnerable to addiction relapse (Monroe & Hadjiyannakis, 2002; Sinha, 2008; Vgontzas et al., 1998), have sleep disturbance or symptoms of insomnia (Vgontzas et al., 1998), and have problems with cognitive functioning in both the short and long term (Chen & Baram, 2016; Stawski, Sliwinski, & Smyth, 2006). In the short-term, preoccupation with earlier stressors can lead to reduced ability to allocate attention and memory resources towards tasks at hand, and long-term chronic stress is associated with accelerated cognitive declines (Scott et al., 2015). Daily stress is further associated with declines in both an individual's health and mood (DeLongis, Folkman, & Lazarus, 1988). Such a broad range of negative and potentially long-term

consequences of stress, and a fast-paced modern world, highlights the need to improve our understanding of how stress impacts our behavior, health, and biological systems.

3. Psychobiological Stress in the Laboratory: The Trier Social Stress Test

In the laboratory, the study of acute stress in humans, through a reliable and valid acute stressor, is essential for basic and translational research (Allen et al., 2014). The TSST (Kirschbaum et al., 1993) is one of the most widely used research tools for the induction of acute psychobiological stress in experimental research worldwide. In its original form (Kirschbaum et al., 1993), the TSST provides an ecologically valid stressor that elicits moderate acute stress through exposure to a psychosocial stressor. It consists of three main components: an anticipation period, a 5-minute mock job interview, and a 5-minute surprise mental arithmetic task; the interview and arithmetic task are performed in front of a panel.

Participants are unaware of the arithmetic task and therefore the TSST contains an element of deceit and uncontrollability. A Dickerson and Kemeny (2004) review determined that motivated performance on tasks containing the central elements of both social evaluative threat (e.g., performance on task is open to negative evaluation by others) and uncontrollability, were associated with the highest cortisol responses and lengthiest recovery periods. As such, the TSST is advantageous in having both of these elements embedded within the protocol, enabling researchers to examine the biological and psychological acute stress responses of individuals within the laboratory (Allen et al., 2017). Deceiving participants is commonly used in psychological research, although this is dependent on the discretion of the individual ethics committees. Certain conditions are necessary for deceit to be acceptable in psychological research, including the condition that the study will make a valuable contribution to the existing scientific understanding, that the deception is not expected to cause significant, ongoing and/or severe harm or emotional distress to participants, and that participants are debriefed about the protocol as soon as the study protocol permits (Boynton, Portnoy, & Johnson, 2013); also see (Hertwig & Ortmann, 2008).

Most commonly in experimental research, acute psychological stressors are measured through assessment of physiological changes, such as an increased HPA axis activity observed through cortisol release (Kirschbaum et al., 1993). Cortisol measurement, obtained from either saliva or blood sampling, has proven to be the most commonly used marker of stress (Goodman et al., 2017). However, a strong stress response following participation in the TSST can be observed via numerous other physiological and psychological markers of stress. This includes significant changes in norepinephrine, epinephrine, salivary amylase, cardiovascular functions, such as heart rate and blood pressure, and electrodermal activity, such as that generated by sweat glands (Miller & Kirschbaum, 2013), increased autonomic nervous system activity (e.g., heart rate variability; Xhyheri, Manfrini, Mazzolini, Pizzi, & Bugiardini, 2012), impacts on the immune system (e.g., increased immune molecules/circulating inflammatory markers following exposure to stress; Steptoe et al., 2007), and changes in gastric function activity (e.g., exacerbating symptoms of irritable bowel syndrome; Kennedy, Cryan, Quigley, Dinan, & Clarke, 2014).

Psychologically, the TSST has also been shown to influence cognitive functions. Specifically, the TSST has been shown to impair working memory for neutral stimuli in those who responded with high cortisol levels (e.g., using a digit span memory task; Elzinga & Roelofs, 2005) and to moderate more complex cognitive functions such as cognitive flexibility (Plessow, Fischer, Kirschbaum, & Goschke, 2011) and creativity (Akinola & Mendes, 2008). Emotionally, the TSST can lead to increased self-reported stress, anxiety, and negative mood (Allen et al., 2014). Administration of the TSST also results in participants reporting increased wakefulness (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999).

While the effectiveness of TSST to induce physiological and psychological changes is clear, research has revealed there are considerable intra- and inter-individual variations in the psychobiological stress response elicited. Demographical, environmental and physiological factors, such as age (Kudielka et al., 2007), sex (Kirschbaum, Wust, & Hellhammer, 1992), education (Fiocco, Joober, & Lupien, 2007), personality (Oswald et al.,

2006), nicotine, alcohol and caffeine consumption (Kudielka et al., 2007), culture (Laungani, 1993), genetics (Ising & Holsboer, 2006), consumption of substances and some medications (see Brody, Preut, Schommer, & Schürmeyer, 2002; Fries, Hellhammer, & Hellhammer, 2006), and methodological aspects (Zänkert, Bellingrath, Wüst, & Kudielka, 2018) are known to impact both the magnitude and the course of the biomarkers examined during stress research. The above factors also likely contribute to both the intra- and inter-individual variations observed in stress response patterns during the TSST (Allen et al., 2014; Miller & Kirschbaum, 2013).

Methodologically, there is notable variability in how the TSST protocol is administered across different laboratories. This is largely due the relatively brief methodological description of the TSST protocol in the original paper using it (Kirschbaum et al., 1993), and because many studies to date have failed to report their specific methodological in detail. Since the publication of the original TSST study in 1993, several attempts have been made to provide an update to the protocol and to review the empirical evidence on the TSST (e.g. Allen et al., 2014; Allen et al., 2017; Birkett, 2011; Goodman et al., 2017). In particular, the impact of variations on the TSST protocol was shown in Goodman et al.'s (2017) meta-analysis of 186 individual studies, which observed differences in effect sizes of the cortisol stress response when different methodological elements were employed across studies. Some of these variations included the time of day administered, sex of participant and other inter-individual differences, number of salivary cortisol measurements and method, panel composition, assessments completed before and during the TSST, speech anticipation times, subtraction number used in arithmetic task, and panel instructions and feedback. Therefore, despite clear evidence for the efficacy of the TSST in experimental research, the field has experienced various inconsistencies in its application and there is limited information available on the exact steps and setup of the TSST.

Developing a widely available and informative TSST guide is one way to reduce some of the intra- and inter-variability observed across studies, while also providing an accessible time-saving resource for those in the field of stress research. Such improvements in

methodological aspects will significantly advance our understanding of how stress impacts health outcomes by providing a better foundation from which to compare findings and in turn advance research in this area.

4. Rationale for an Introductory Guide to the TSST

In light of the apparent lack of a consistent and detailed description of the TSST methodology, the aim of this paper is to provide an overview of the methodological inconsistencies in the administration of the TSST, and more importantly, to provide an introductory step-by-step guide in the form of a laboratory manual as a foundation for researchers to use when developing and administering the TSST. We focused only on the administration of the TSST to individual adult participants. This includes the specific components of a waiting period, task introduction, anticipatory phase, speech period, surprise arithmetic task, debrief, and recovery as per the original protocol (Kirschbaum et al., 1993). We acknowledge that there are various versions of the TSST, such as the group version (TSST-G; von Dawans, Kirschbaum, & Heinrichs, 2011), the virtual reality version (TSST-VR; Kotlyar et al., 2008), a version suited to children (TSST-C; Buske-Kirschbaum et al., 1997), a control (placebo) version of the TSST (Het, Rohleder, Schoofs, Kirschbaum, & Wolf, 2009), a friendly TSST (f-TSST; Wiemers, Schoofs, & Wolf, 2013), a modified version suited to both adults and children (TSST-M; Yim, Quas, Cahill, & Hayakawa, 2010) and an electronic version (e-TSST; Hawn, Paul, Thomas, Miller, & Amstadter, 2015) published elsewhere.

Here, we aimed to establish the shortest possible protocol and guide of the TSST for adult participants that considers participant burden and ease of application. Our approach has been to not only collate existing details of current research and reviews of the literature, but to also integrate this with details from our own experiences that are absent from the original protocol and that have appeared necessary to conduct the TSST (e.g., a glucose drink to account for inter-individual variation in baseline blood glucose levels). Our personal recommendations have all been in consultation with experts in the field (see

Acknowledgements) and based on existing evidence and theoretical knowledge. In some instances, we have highlighted were large effect sizes for some elements, such as using a three-person (vs. two-person) panel (Goodman et al., 2017), may prove practically difficult to implement in real experiments and may therefore not the best choice. Moreover, these introductory guidelines may also be purposefully manipulated to test certain outcomes. For example, one may wish to explore the impact of acute stress on cognitive functioning by administering a cognitive battery during baseline, in anticipation of stress, immediately post-stress and after a period of rest during the TSST (e.g., Olver, Pinney, Maruff, & Norman, 2015). We anticipate that this introductory guide will assist in establishing a scientific standard that can improve the rigor and reliability of the reported stress response in human research. However, such a guide provides only a foundation and by no means represents binding guidelines that all researchers must adhere to.

In the next section, we discuss the core elements of the TSST. We also provide suggestions, where empirically supported and suitable for the individual research question, to consider when employing this protocol and adapting it to the individual study.

5. A Guide to Conducting the TSST

In *Figure 1*, we provide an overview of procedures for conducting the TSST. Briefly, a minimum of three investigators are required to run the protocol; one investigator to assume the role of the lead researcher who will oversee the entire protocol and remain with the participant from arrival to departure, along with two panel members who are present for the active component of the TSST. Two testing rooms are also required; see ***Supplementary material*** for illustrations.

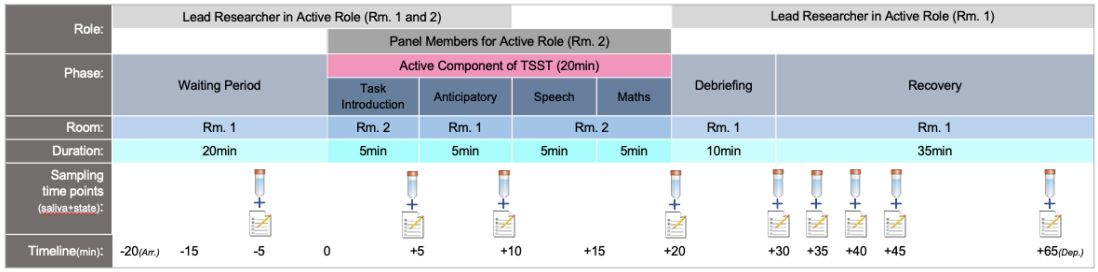


Figure 1. An illustration of the various phases of a standardized TSST protocol. The top row (Researcher Roles) depicts the presence of each researcher for the separate Phases and Rooms of the TSST. The second row (Phase) depicts the separate phases involved in the TSST from the beginning to end. Below the Phases, the Room row depicts where each of these phases takes place, i.e., Room 1 (Rm.1) being the waiting room, and Room 2 (Rm.2) being the TSST room. The Duration row includes the Duration, and depicts, in minutes, the length of each of the separate phases of the TSST. The red box in the duration row indicates the active components of the TSST and below this the TSST timeline is included. The TSST timeline is as per the original protocol (where time point 0 min indicates the onset of the active TSST, i.e., task instruction), with the participant arrival occurring at -20min to TSST onset. As per the suggestions of this manuscript, the departure occurs at +65min. Lastly, below the recommended TSST timeline, the figure includes the suggested timing of 9 sampling time points for cortisol and state psychological assessments, structure around the expected peak cortisol response.

The participant arrives to the TSST testing session 5 minutes prior to beginning the waiting (acclimation) period. The participant is introduced to the waiting room (Room 1) and its purpose throughout the laboratory session, and completes any required paperwork, after which the participant is directed to consume a glucose drink and rinse their mouth of any residue with a standardized allotment of water (100 milliliters). Immediately following this, the participant commences the waiting period (15 minutes until TSST onset; the 20-minute waiting period in Fig. 1 is inclusive of the 5 minutes for study particulars (e.g., arrival and consent), along with 15-minute acclimation). At time point 0, the TSST onset, the participant is led to the second room (Room 2) to begin the 5-minute task introduction period; during this time the participant enters Room 2 to face the awaiting panel (2 members). At this point, they are introduced to the task they will complete via a standardized script read aloud by the lead researcher. The participant is then returned to Room 1 where they begin the 5-minute anticipatory stress period, during which they prepare their speech for the mock job interview as per the instructions provided in the task introduction (excess time remaining from the 5-minute task introduction period allows for transport to and from Room 1 and Room 2, and for completion of sampling during this period). The participant is then returned to Room 2 at +10 minutes where the lead researcher, while in view of the participant, begins the video recording. The researcher then proceeds to leave the room before the participant commences

their 5-minute speech portion of the task (the active panel member will provide instructions to start their speech). This action of beginning the video recording, observed by the participant, is an important element of the TSST that largely contributes to the social evaluative component of the stress response. At the conclusion of the speech period (+15 minutes) the active panel member introduces the participant to the 5-minute surprise arithmetic task to be completed for the remaining 5 minutes of the active component of the TSST. At the conclusion of the arithmetic task (+20 minutes since TSST onset) the participant is returned to Room 1 for sampling, followed by a debriefing period, before then remaining in Room 1 for the recovery period.

It should be noted here that this paper and guide focus specifically on salivary cortisol sampling for measuring the biological stress response as it is a practical and cost-effective biomarker measurement, with minimal participant burden; for advantages of different biomarkers of the TSST response, see Allen et al., (2014). As per the original protocol (Kirschbaum et al., 1993), an alternative collection method to measure the physiological (cortisol) response to the TSST is blood sampling through venipuncture. However, in contrast to serum or plasma which allows only total cortisol measurement, salivary cortisol analysis represents the unbound biological active cortisol that are independent of flow rate and which lags behind serum levels, thereby presenting a better assessment of baseline cortisol that is not confounded by stress related to the environment, such as the venipuncture or meeting the researcher (Kirschbaum & Hellhammer, 1994). Given that the process of venipuncture can lead to increased psychological and physiological distress (i.e., from fear of the needle), a longer acclimation period, such as a 45-minute waiting period, may also be needed post venipuncture to allow for any heightened stress response to the venipuncture to return to baseline. That said, in their meta-analyses, Goodman et al. (2017) found that studies using intravenous catheters showed the highest cortisol stress response across all protocol variations. Thus, it is unclear if a longer waiting period is needed and whether the actual cortisol response is compromised. Regardless, the blood sampling time points for venipuncture can continue as per the recommendations outlined in *Figure 1*.

In the following sections, we discuss suggestions for conducting the TSST and considerations for adapting and varying the components of the TSST.

5.1 Timing of Administration

5.1.1 Time of day

The diurnal pattern of cortisol secretion in typically healthy adults exists in two components; a sharp increase in cortisol levels following awakening, known as the Cortisol Awakening Response (CAR) that is seen within the first hour post waking, and a subsequent decline in free cortisol concentration across the rest of the day (Edwards, Evans, Hucklebridge, & Clow, 2001). Additionally, increases in cortisol are seen following the consumption of a meal (Kirschbaum et al., 1993), whereas a hypoglycemic state prior to the consumption of a meal has been associated with a decreased cortisol stress response (Davis, Shavers, Davis, & Costa, 1997). Given the fluctuations in normal (baseline) cortisol levels during the day, it is suggested that the TSST is conducted consistently at a set time that best reduces the systematic impact of these factors on the cortisol stress response to minimize inter-individual variation in baseline cortisol levels amongst participants.

With the expectation and understanding that baseline cortisol levels decrease as the day progresses, the afternoon has typically been regarded as the preferential TSST start time. Further, in consideration of post-meal cortisol increases and pre-meal hypoglycemia resulting in reduction in cortisol stress response, mealtimes are typically avoided (e.g., large meals should be avoided within one hour of the TSST). An opportune time window is after lunch and before dinner, which capitalizes on the natural low levels of cortisol concentration in the afternoon and avoids the influence of food consumption on cortisol levels and stress response. Recently, Goodman et al. (2017) determined in their meta-analysis that, for studies with TSST occurring in the A.M. period (morning: $n = 17$; Cohen's $d = 0.784$) or during the lunch-time period (12pm – 2pm: $n = 30$; $d = 0.811$), the cortisol stress response strength was slightly lower and exhibited more variability when compared to studies where the TSST took place between 2pm – 5pm (afternoon: $n = 42$; $d = 0.962$). In consideration of the above, the TSST

should be conducted within a time period that factors in the diurnal pattern of cortisol secretion (e.g., avoiding the CAR period and periods of higher cortisol concentration at the beginning of the day/post-awakening) and that avoids pre-meal slumps in cortisol stress response and post-meal increases in cortisol levels. Other start times (e.g., late morning) may still be considered feasible alternatives, particularly for studies utilizing the TSST for purposes other than cortisol stimulation (e.g., sensor data such as galvanic skin response, blood pressure, heart rate, or psychological state data) but consideration should be paid to the pattern of cortisol secretion across the day and the impact of meals in proximity to testing. Ideally, timing of the TSST protocol should be kept consistent within a single experiment.

5.1.2 Waiting and speech anticipation time

External stressors unrelated to the individual's participation, or the participation in a research study itself, raise the possibility that participants may arrive at the laboratory in an already anxious state, such as from rushing to get to their appointment on time. To avoid elevated baseline cortisol levels prior to the TSST, study protocols typically include a waiting or acclimation period. Allowing time for a participant to acclimatize and recover from prior stressors, is necessary in facilitating a return to baseline (pre-study) cortisol levels (Dickerson & Kemeny, 2004).

The original protocol (Kirschbaum et al., 1993) outlined a participant acclimation period of 10 minutes (or 30 minutes for blood sample), with a speech anticipation period of 10 minutes. The Goodman et al. (2017) meta-analysis showed that studies with a 16-30 minute acclimation period had the highest effect size (n = 67; d =0 .966), but which decreased as the waiting durations increased to 31-60 minutes (n = 65; d = 0.889) and beyond 60 minutes (n = 33; d = 0.799). As it stands, the ability of the TSST to produce cortisol stress responses has proven to be robust and ideally the overall protocol should aim to minimize participant burden and improve logistic operations and convenience. As such, it is suggested the participant should arrive 20 minutes prior to TSST onset (time point 0). This allocation allows 5 minutes for appropriate introduction and set-up, accompanied by a 15-minute acclimation period.

Regarding the speech anticipation period, the meta-analysis (Goodman et al., 2017) revealed similar mean effect sizes across studies with 3, 5, or 10-minute periods (*ds* = 0.891, 0.904, and 0.947, respectively). This is despite the original protocol allowing 10 minutes (Kirschbaum et al., 1993). Given the comparable effects, the speech anticipation period is one TSST element that can be kept relatively short to reduce the overall burden and improve logistics (Goodman et al., 2017). A 5-minute speech anticipation period may be most ideal to allow adequate time for any sampling to occur, while also providing sufficient preparation time for the participant.

5.1.3 Reduction in overall protocol time

The original TSST protocol operated across a range of 60-120 minutes, dependent upon the number of cortisol sampling collections and type of sampling, e.g., blood or saliva (Kirschbaum et al., 1993). In *Figure 1*, we present a TSST protocol with a testing period of 85 minutes that we believe is the shortest and most practical length. This is inclusive of: arrival and acclimation (5-minute arrival; 15-minute acclimation), task introduction (5 min), speech anticipation (5 min), speech portion (5 min), arithmetic portion (5 min), debrief (10 min), and recovery (35 min). These time periods are consistent with the original protocol, except for the length of time for the task introduction period, which had no specified time. Here, we propose a standardized length of time of 5 min for the introduction period for consistency across studies. In addition to the task introduction itself, this 5-minute allocation allows time for the participant to walk between the two testing rooms (i.e., participant goes to Room 2 to receive the task introduction after which they return to the initial waiting room), address any questions or concerns, and complete any required sampling before commencing their 5-minute speech anticipation period.

The most time-dependent element is the number of biomarker sampling points throughout the protocol. Peak salivary cortisol levels are reported to occur 10 minutes post cessation of the active stress, i.e., 10 minutes post conclusion of the surprise arithmetic task (Kirschbaum et al., 1993). Results from the Goodman et al (2017) analyses determined that the highest cortisol levels typically occurred between 35-45 minutes post TSST onset. In light

of the peak salivary cortisol levels observed in the original study (Kirschbaum et al., 1993) and current evidence from the meta-analysis (Goodman et al., 2017), frequent saliva sampling from time point 30-45 minutes after TSST onset (i.e., minimum of 4 collections; see *Figure 1*) is suggested to capture the full extent of the salivary cortisol response post-acute stressor.

Following the gradual peak in cortisol post-TSST, a gradual decline in cortisol levels is expected. The recovery period of 35 minutes, which includes samples from 30-65 minutes after the onset of the task (*Figure 1*), is enough time for the peak cortisol stress response to be captured and for the cortisol levels to begin returning to baseline levels. However, if a complete return to baseline (pre-stimulation basal diurnal levels) values of cortisol is of particular interest, then the recovery period should be extended to at least 60 minutes (i.e., 90 minutes post onset of the TSST), resulting in an overall testing period of 110 minutes (Kirschbaum et al., 1993).

- ***Summary:*** An afternoon (e.g., between 2pm – 5pm) scheduling of the TSST may minimize inter-subject fluctuations in baseline cortisol levels. Other times may be viable, so long as researchers factor meal consumption and patterns of cortisol secretion into the chosen timing of administration of the TSST. Moreover, a 20-minute pre-TSST onset arrival is suggested, allowing for 5 minutes of introduction and set up, followed by an acclimation period of 15 minutes. A 5-minute speech anticipation period is suggested. For studies using saliva sampling, the protocol length may be reduced to 85 minutes total duration to reduce participant burden while capturing the peak cortisol stress response levels and some recovery. Should capture of return to basal diurnal levels of cortisol be desired, the duration of the recovery phase should be increased from 35 to 60 minutes resulting in 110 minutes total duration. Timing of the protocol should be kept consistent within a single experiment.

5.2 Participants

5.2.1 Physiological and environmental factors

As previously stated, considerable intra- and inter-individual variations in the psychobiological stress responses from the TSST have been observed due, in part, to a range of environmental and physiological factors. These factors should be considered when screening a participant for the TSST and on the day of the TSST. Age and gender are known factors influencing the stress response, with the impact of genetic and cultural factors also requiring consideration (Allen et al., 2014). Sleep cycles may also impact on salivary cortisol results, as such deviations from the expected sleep routine, such as night shift or insomnia, should be screened for. Some medications (e.g., oral steroids) also result in blunted or false salivary cortisol results and should be excluded from sampling.

Also as previously noted, in addition to the normal diurnal variation, cortisol levels in the body are known to rise after each meal (Follenius, Brandenberger, & Hietter, 1982; Quigley & Yen, 1979). Therefore, participants should avoid consuming substantial meals/food or beverages other than water in the hour leading up to the TSST. Additionally, no vigorous exercise should occur within the hour prior to the TSST, as there is evidence showing that moderate to high intensity exercise can provoke an increase in circulating cortisol levels (Hill et al., 2008). A record of these details should be kept for each participant so that potential variations may be accounted for where relevant.

5.2.2 Menstrual cycle and sex hormones

Research has shown large gender variability in the salivary cortisol response to acute stressors, including in response to the TSST, when no appreciable differences in cortisol levels were noted in the pre-stress levels between genders (Kudielka, Hellhammer, & Wust, 2009). This is because sex hormones differentially affect salivary cortisol response (Lennartsson, Kushnir, Bergquist, Billig, & Jonsdottir, 2012; Stephens, Mahon, McCaul, & Wand, 2016). Specifically, for healthy adults the salivary cortisol response to acute stress in the laboratory is significantly larger for men than women (Earle, Linden, & Weinberg, 1999;

Lovallo, Farag, Vincent, Thomas, & Wilson, 2006; Nicolson, Storms, Ponds, & Sulon, 1997; Seeman, Singer, Wilkinson, & McEwen, 2001; Steptoe, Fieldman, Evans, & Perry, 1996).

Moreover, there is also evidence of female menstrual cycle phase significantly influencing cortisol levels to psychosocial stressors. As evidenced by Kirschbaum et al. (1999), women completing the TSST during the luteal phase of their menstrual cycle (between 14 to 28 days since the first day of their last menstruation of a regular 28-day cycle), produced salivary cortisol responses comparable to males, while women in the follicular phase or women using hormonal contraceptives exhibited significantly lower salivary cortisol responses (Kirschbaum et al., 1999). The same study also found that women on oral contraceptives produced lower cortisol response to the TSST (Kirschbaum et al., 1999). Others found that, compared to women in their follicular phase of their cycle, those in the luteal phase showed significantly higher cortisol levels following the TSST-VR (Montero-Lopez et al., 2018), although this finding was in contrast to that of another study (Maki et al., 2015).

It would therefore be ideal to control for contraceptive use and menstrual cycle phase in experiments involving women. Future studies need to determine which phase of the menstrual cycle make women more comparable to men, although some evidence is suggesting that this is the luteal phase (Kirschbaum et al., 1999). For an in-depth review of the potential influences of gender, endogenous sex steroids, menstrual cycle phase, oral contraceptives and corticosteroid binding globulin on the salivary cortisol response to stress, see Kudielka and Kirschbaum (2005) and Villada et al. (2017).

- ***Summary:*** It is recommended that females are free from contraceptives and tested during the same menstrual cycle phase, e.g., luteal phase. However, this may be manipulated depending upon the research question. At minimum, a record of menstrual cycle phase and of any hormonal contraceptives at the time of the TSST should be kept. Moreover, determining the timing of phases in female participants can be difficult and thus researcher may want to consider doing a urine luteinizing hormone kit as a more objective measure.

5.3 Salivary Cortisol Sampling

5.3.1 Collection time points

Across TSST studies, there are large variations in both the number of saliva samples collected and the time points at which these samples are collected. The profile of the cortisol response from the stressor indicates a gradual rise, rather than a peak, that is followed by a gradual fall in cortisol values (Dickerson & Kemeny, 2004). The original TSST paper reported peak salivary cortisol levels at time-point +30 min post the onset of the TSST (i.e., time-point 0 being the task introduction), therefore 10 minutes after the cessation of the arithmetic task (Kirschbaum et al., 1993). Results from the Goodman et al. (2017) analyses determined that the highest cortisol levels typically occurred between 35-45 minutes post the onset of the TSST, therefore 15-25 minutes after the cessation of the arithmetic task. Considering this, frequent sampling between 30 and 45 min after TSST onset is suggested (e.g., +30; +35: +40; +45; see *Figure 1*) to ensure the full extent of the salivary cortisol response post-acute stressor is captured.

In their meta-analysis (Goodman et al., 2017), the majority of TSST studies sampled an average of 4.1 samples in the 30 min after the cessation of the arithmetic task. The total number of samples collected depends on the research question and need to consider cost and participant burden. In *Figure 1*, a maximum of 9 samples is depicted to illustrate potential sampling time points to capture baseline cortisol level, gradual cortisol stress response, and a return to baseline. Alternatively, as little as 2 samples may suffice for some research questions (e.g., a manipulation check in a cognitive study). Where the profile of cortisol response is of interest, we suggest 5-6 samples at a minimum, including one collection at pre-stress post acclimation baseline (time point -5), 3-4 collections occurring after the TSST onset to best capture the gradual rise and fall of the peak cortisol response (+30, +35, +40, +45), and a final recovery collection (~ +90 min post TSST onset) to capture a return to baseline cortisol levels.

5.3.2 Salivary collection method

There are various commercially available methods for collecting saliva that include passively drooling saliva into a tube, or absorbing saliva onto a device such as an oral swab (e.g., SalivaBio swabs from Salimetrics) (Rohleder & Nater, 2009) or a salivette (Sarstedt). These collection methods are minimally invasive, do not require specialized skills, and are convenient for repeated collections. For the TSST, the absorption method (e.g., using the commercially available synthetic oral swabs) is recommended as both a hygienic and a practical tool that comes with greater ease of use for multiple collections so long as only a small volume of saliva is of interest, i.e., to analyze cortisol levels only. Participants are required to place the oral swab in the mouth positioned under the front of the tongue, which is then left there for 1-2min to absorb the saliva. The saliva volumes are then extracted by centrifuge.

However, where additional analytes are of interest, the passive drool method (e.g., SalivaBio, Salimetrics) will be more appropriate as it collects a larger volume of saliva. This method is approved for use with nearly all analytes and considered the gold-standard for collecting whole saliva. It requires participants to passively pool saliva at the bottom of the mouths and to expel this into a collection device, such as a plain tube with or without a straw.

For the measurement of cortisol specifically, both the absorption and the passive drool methods have been widely used. However, there is evidence that the collection method significantly influences the accuracy of cortisol measurement in saliva (Gallagher, Leitch, Massey, McAllister-Williams, & Young, 2006). The use of the absorption method with salivettes has been found to be a more reliable predictor of total and free serum cortisol than passive drool (Poll et al., 2007). In the latter study, both participants and technical staff also preferred the absorption method for saliva collection. Of note, salivettes produced lower cortisol concentration levels compared to the passive drool in the latter study (Poll et al., 2007), which is in line with evidence that cotton can reduce cortisol levels and create random errors (Shirtcliff, Granger, Schwartz, & Curran, 2001; Strazdins et al., 2005). There are also

variations in analyte levels depending on the positioning of the absorbent device that may result in collection of localized saliva rather than whole saliva (Granger et al., 2007).

Our recommendation of using oral swabs (e.g., SalivaBio; Salimetrics) for the multiple collection time points in the TSST relates to these devices providing a potentially quicker collection (as only small volume is required) and because oral swabs are synthetically made from a non-toxic inert polymer, to eliminate the variable and inconsistent results demonstrated through the use of a biologic material, such as cotton (Shirtcliff et al., 2001). Researchers may also opt to conduct a pre- and a post-TSST saliva collections using the passive drool method to provide a larger volume suitable for additional analytes, such as measurement of other hormones or metabolites over only two sampling time points. Ultimately, care should be taken when comparing studies that used different salivary cortisol collection methods. It is vital that all studies carefully report the collection information, such as the method of collection including the type of swabs, location of these in the mouth, and duration of collection.

5.3.3 Glucose drink and baseline saliva

Details about the inclusion of a glucose drink in the TSST protocol were omitted from earlier descriptions of the protocol including the original study (Kirschbaum et al., 1993). This methodological detail has not been explicitly outlined in any of the previously published TSST protocols (Birkett, 2011; Kirschbaum et al., 1993). The inclusion of the glucose drink has only been included in studies where the researchers were intuitively aware of this addition and its purpose (e.g., through word of mouth). Glucose loading prior to the TSST is thought to account for inter-individual variation in baseline blood glucose levels prior to testing, which aids in the production of a reliable cortisol response curve when compared to water (Kirschbaum et al., 1997).

Evidence suggests that the response of the HPA axis is closely related to the physiological systems that are responsible for caloric movement and energy availability within the body (Dallman et al., 1993). To explore the impact of glucose levels and subsequent caloric loading on the free cortisol response to acute stress, Kirschbaum et al.

(1997) manipulated the blood glucose levels of participants prior to inducing acute psychosocial stress. For healthy individuals with low glucose levels, an inhibited adrenocortical response was observed to the TSST, while those with high blood glucose levels displayed the expected doubling of the amount of free cortisol in the system following the stressor (Kirschbaum et al., 1997). Thus, readily accessible energy, achievable through the recommended glucose loading prior to stimulation, is recommended as a pre-requisite for a strong HPA stress response (Kirschbaum et al., 1997) and in line with animal studies (Akana, Strack, Hanson, & Dallman, 1994; Hanson et al., 1994). Depending on the research question however, some researchers may opt to omit the glucose drink, for example, if there is a specific research question about normal/baseline variations between certain groups of interest. Following consumption of the glucose drink (to be administered at the beginning of the waiting period), it is imperative that participants use a standardized allocation of water to rinse their mouths and to remove any glucose drink residue to not interfere with saliva sampling; see ***Supplementary materials***.

- ***Summary:*** The number of samples to be collected is dependent upon the specific research question and the affordability of sampling. The proposed 9 collections allow for an optimal number of samples to capture the full cortisol response profile. Oral swabs are practical and relatively easier to use for multiple cortisol collections, but are limited in saliva volume, and may need to be supplemented with passive drool sampling (e.g., at two sample time points, pre- and post-TSST). Careful adherence to procedures to avoid contamination of samples must occur, along with a detailed description of the collection method in future studies. The inclusion of glucose loading via a glucose drink needs to be considered in light of the research question.

5.4 Panel Arrangements

5.4.1 Age, sex and number of panel members

Since the protocol's conception in 1993, a number of variations from the original protocol have been introduced into the social interaction component of the TSST.

Specifically, there have been variations in the number of panel members, the age of panel members, the gender composition of the panel members, the age and sex matching of the active panel member to the participant, and the level of interaction between the panel members and the participant.

Regarding the gender composition of the TSST panel, psychological and neuroendocrine studies suggests that interactions with the opposite gender result in increases in anxiety and discomfort for both men and women (Chorney & Morris, 2008; McCubbin et al., 1991). In relation to the TSST, both men and women (in the follicular phase) present with greater cortisol increases from the acute stressor when exposed to panel compositions that include the opposite sex (Duchesne, Tessera, Dedovic, Engert, & Pruessner, 2012). Meta-analyses revealed that an all-female panel resulted in one of the lowest effect sizes for cortisol stress response strengths (n = 11; d = 0.547), with a mixed-gender panel eliciting cortisol stress responses of a very large effect (n = 146; d = 0.975) (Goodman et al., 2017). Although there was no evidence on an all-male panel, a mixed-gender panel may serve the best option given the known evidence of heightened reactivity in men and women to the opposite gender.

What is as yet unclear is whether the active panel member needs to be gender-matched, cross-gender matched, or randomly allocated. The majority of the TSST studies have not reported this information (Goodman et al., 2017). Given the evidence that men and women have heightened reactivity, such as cortisol increase, when presented with the opposite gender, it may be feasible to suggest that the active panel member is cross-gender matched (e.g., male participant and female active panel member). However, strong recommendations of the gender orientation of the active panel member cannot be made as more research is required. It is recommended that future research studies explicitly report such details. This is particularly important as there are significant differences in the reactivity of men and women's stress responses during social interactions (Verma, Balhara, & Gupta, 2011) that requires further examination.

Although there is evidence on the ageing effects of cortisol reactivity to an acute stress, such that older adults respond with lower cortisol responses to the TSST (Hidalgo et

al., 2015), few studies comment on the age of panel members. To date, there is no evidence on the potential impact of the age of panel members on participants' cortisol reactivity during the TSST. It is recommended future research records the ages of panel members to determine whether the age of panel members may also impact the observed cortisol stress response. Given evidence of own-age bias in face processing (Ebner & Johnson, 2010), it is possible that age concordance between participant and panel members (e.g., young participant and young panel members) may result in relatively lower cortisol reactivity compared to an age discordance (e.g., young participant and old panel members). However, the latter remains to be empirically tested.

Regarding the number of panel members, the original TSST protocol recommended three members (Kirschbaum et al., 1993). However, ff the 186 studies analyzed by Goodman et al. (2017), large effects were obtained in studies utilized two-member panels (n = 153; d = 0.891). Although lower, these effect sizes were relatively similar to those reported for three-member panels (n = 32; d = 1.064). While a three-member panel may induce a somewhat stronger cortisol stress response, it is more labor-intensive as it requires significant coordination in obtaining volunteers for a three-member panel for each participant that also adheres to the mixed-gender panel composition. Thus, a less burdensome two-member mixed-gender panel may be better suited to most research environments.

5.4.2 Demeanor of the panel member

In the active part of the TSST, the experiences of uncontrollability and social-evaluation are elicited through a mock job interview that is followed by a surprised arithmetic task in front of a panel. The behavior of the panel toward the participant is a key element for contributing to a participant's feelings of uncontrollability and threat to self. A Dickerson and Kemeny (2004) meta-analytic review of over one hundred stress studies, determined that elements of uncontrollability and threat to the social self and self-esteem (i.e., social-evaluative threat) exhibit the greatest efficacy for inducing significant increases in cortisol stress responses, with these elements incorporated into a number of psychosocial stress

paradigms, including the TSST. The significance of these factors was subsequently tested when a control (placebo) version of the TSST was introduced by Het et al. (2009). The placebo protocol was similar to the original TSST protocol but with the removal of uncontrollability and social-evaluative threat (i.e., no panel members and no video recording), resulting in a significantly reduced salivary cortisol response (Het et al., 2009).

As per the original protocol (Kirschbaum et al., 1993), the recommendation remains that all panel members present with a strictly neutral demeanor toward the participant during the active component of the TSST (speech and arithmetic tasks) as the panel members take on the role of evaluating the participant's performance without the provision of any signs or indications of social support or cues. This neutral demeanor of the panel members contributes to the participant's experience of *uncontrollability* and *threat to the social self and self-esteem*, as well as the successful activation of the HPA and sympathetic nervous system (e.g., Kirschbaum et al., 1999).

Meta-analyses of 131 studies determined that cortisol stress responses were significantly lower for the studies in which the panel explicitly provided negative feedback to participants ($n = 24$, $d = 0.713$) as oppose to neutral feedback ($n = 107$, $d = 0.869$) (Goodman et al., 2017). It is possible that the ambiguity of a neutral expression contributes to higher cortisol stress response. We commonly deal with positive feedback and interactions in daily life, and have become accustomed to handling negative feedback or criticism (Swann, Wenzlaff, Krull, & Pelham, 1992). It is additionally important that panel members ensure they do not unknowingly display negative mannerisms to the participant. Thus, panel members should be engaged with the participant, such as by showing interest in the participant's speech, but should not demonstrate a stone-cold manner that could be interpreted as negative.

5.4.3 Appearance

For consistency and to emulate a professional interview environment, it is suggested the panel members and lead researcher wear white lab coats, or work wear that matches the

environment, throughout the entire protocol (except during the debriefing period when the lab-coats are to be removed to encourage a greater reduction in stress levels in the participant). Panel members and the lead researcher should also wear neat professional dress, with appropriate footwear, especially where feet are visible from underneath the panel's table in order to maintain the professional environment.

5.4.4 Panel familiarity

To maintain a convincing interview environment and uphold the integral theme of uncontrollability and social evaluation, participants should be unfamiliar to the panel members. This is particularly relevant in teaching environments where participating students may know the research staff on the panel (e.g., if staff on the panel are also teaching or administrative staff).

- ***Summary:*** A two-member panel that is gender-balanced is ideal. Details of the age of the panel members and whether the gender of the active panel member was matched to the participant should be reported for future studies to consider. All panel members are to remain neutral (but not negative) toward the participant across the active component of the TSST. The panel members are also to wear professional clothing and they should be unknown to the participant.

5.5 Assessments during the TSST

Participants are often required to complete several assessments (task-based and questionnaires) at baseline, prior to the commencement of the TSST. Meta-analyses revealed that the completion of assessments such as questionnaires during the pre-TSST acclimation period produced a slightly lower effect size (n =109; d = 0.823) when compared to a waiting period with no such activity (n = 49; d = 1.031) (Goodman et al., 2017). The number and type of assessments administered pre-TSST, and the potential for these assessments to induce stress in the participant and impact baseline cortisol levels, should be considered as these factors could reduce the overall stress response.

Interestingly, when state version of the State Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) were administered during the speech anticipation period (n = 42; d = 0.846), were comparable to when no questionnaires during this period (n = 62; d = 0.849) (Goodman et al., 2017). Therefore, completing assessments at a later stage, such as during the speech anticipation period rather than waiting period, may have the least amount of influence on the cortisol stress response. We suggest that an ideal time to administer a brief psychological assessment (e.g., the STAI) would be at the end of the acclimation period or immediately following the task introduction, but before the speech anticipation period.

These state assessments could also be repeated during the post-test recovery period to provide a measure of changes in state anxiety across the duration of the TSST. Additionally, included alongside each cortisol sampling time-point, a brief state assessment may be repeatedly administered in order to provide a subjective assessment of the participant's experience during the TSST. For example, participants can be asked to rate the way they feel at the time using a short visual analogue scale assessing dimensions of happiness, sadness, tiredness, anxiety and withdrawal; see ***Supplementary material*** for an example of a visual analogue scale.

The type and number of assessments to be administered during the TSST is at the discretion of the researcher and the specific research question, but these should be considered in light of the potential burden of any assessments on the participants. Assessments with minimal demands and impact on the mood, such as brief visual analogue scales (Aitken, 1969), may be preferred over those where sensitive information, such as traumatic life events, is assessed.

- ***Summary:*** If assessments are utilized pre-TSST, these should be administered towards the end of the acclimation period or alternatively immediately following the task introduction prior to the speech anticipation phase. Selected assessments should require minimal demand and have little impact on the mood of the participant but may vary

depending on the research question. A subjective assessment of the participant's experience during the TSST should be collected.

5.6 Other Psychobiological Markers: Heart Rate

In addition to cortisol measures, some researchers may be interested in obtaining other psychobiological markers of stress, such as heart rate and heart rate variability. Heart rate was measured in one of the studies included within the original TSST protocol (Kirschbaum et al., 1993); this was obtained while the participant was standing in front of the panel (this detail was unspecified in the original protocol). There is evidence to suggest that body position, such as prone, supine and sitting, significantly affects blood pressure and heart rate (Watanabe, Reece, & Polus, 2007). For example, blood pressure was higher and heart rate was lower in the prone (vs. sitting) position. It is recommended that researchers interested in the recording of heart rate note the position of the participant during the active component of the TSST and collect a baseline measure of heart rate accordingly. For example, if participants are standing during the active TSST (speech and arithmetic task), it is ideal to also obtain an equivalent standing baseline heart rate measurement (e.g., for 10 minutes) that is collected during the acclimation period (pre-TSST). For a detailed description of other psychobiological markers of stress other than salivary cortisol and heart rate discussed here, see Allen et al (2014).

- ***Summary:*** Other physiological markers of stress, such as heart rate, may be added to the TSST protocol.

5.7 Debriefing

At the conclusion of the speech and arithmetic task, the participant is returned to the waiting room where they are debriefed. During debriefing, the lead researcher informs the participants that the TSST is specifically designed to elicit psychobiological stress inside a laboratory environment, and that the speech and arithmetic tasks were intentionally difficult and do not in any way reflect their aptitude or ability. The prop elements such as the use of the camera should also be explained, along with an explanation that the panel was instructed

to provide no feedback or encouragements during the task. Participants are also told they are not actually evaluated or compared to others' performances on the task, with the actual measurement of interest being their physiological (e.g., cortisol) response. Participants are also given time to ask questions. Following the debriefing and questions, the panel members should be welcomed into the waiting room, with their lab coats removed, so that they may greet the participant. The latter allows for improved debriefing and recovery periods and a return to baseline in cortisol levels (see ***Supplementary material*** for additional details, such as a debriefing script).

- ***Summary:*** Debriefing is a crucial part of the TSST and should contain a detailed exchange of details between the participant and lead researcher, aided by the panel members, to ensure a thorough recovery process. This is also a vital component for managing the element of deception used in the TSST protocol.

6 Limitations

There are limitations to consider in this critical review and introductory guide. Firstly, we did not provide a comprehensive review of the TSST as such detailed descriptions of the key elements and related variabilities has been done elsewhere (e.g. Allen et al., 2014; Allen et al., 2017; Birkett, 2011; Goodman et al., 2017; Kudielka et al., 2007). Instead, we focused on developing and providing the most detailed step-by-step guide and laboratory manual for researcher to use to improve the administration and rigor of TSST research and to provide those unfamiliar to the protocol with significant details. As more research is being conducted, it is likely that some of recommendations will need to be updated. For example, is it unclear whether the age of the panel members and other cultural factors may influence the cortisol stress response. Secondly, we only focused on the individual adult version of the TSST as it is the most commonly used version. Ultimately, detailed guides for other versions of the TSST will also be needed, and our current guide and recommendations provide the foundation for additional detailed protocols to be developed. Thirdly, we focused our attention on only salivary cortisol, with some mention of considerations for heart rate and venipuncture. We

focused on salivary cortisol as it is the most reliable and practical biological marker for stress response to date. However, we acknowledge that there are additional biological markers (e.g., ACTH, vasopressin) and bodily systems (e.g., immune system, cardiovascular system, sympathetic adrenal-medullary system) that may be of interest, but which inclusion of these was beyond the scope of this review and online guide. Relatedly, we did not cover in detail the use and role of subjective measures of stress, however such a measure is included in our guide in the ***Supplementary material***. Finally, we acknowledge that the use of a consistent protocol across the TSST studies is not enough to create more homogenous outcomes as various other factors will still create variability. One such factor is the various assaying techniques across laboratories, whether internally developed or commercial. Other variability may also come from sample characteristics, infrastructure (e.g., rooms), and questionnaires (e.g., in the waiting period). Yet, for researchers interested in conducting the TSST, this introductory guide will provide a useful and time-saving resource that may help improve consistency in the literature.

7 Conclusion

With this paper, we provide an overview of the TSST for adult participants and the use of salivary cortisol measures. Most importantly, we provide a detailed step-by-step introductory guide that presents the shortest possible protocol for TSST administration, taking into account participant burden and practicality (in the ***Supplementary material)***. We also provided informative reference of considerations for adapting the protocol to the individual research question. This paper and introductory guide make an important contribution to stress research as the TSST remains one of the most widely used protocols for inducing acute psychobiological stress in humans. We hope to improve the research standards in the field and to ultimately advance the understanding of how stress impacts everyday functions that have important implications for future health.

For viewing purposes, the supplementary material is presented directly following the manuscript.

5.6 SUPPLEMENTARY MANUAL

Version 1.0

Trier Social Stress Test (TSST): An Introductory Manual

September 2019

Table of Contents

1. Schematic Overview of the TSST

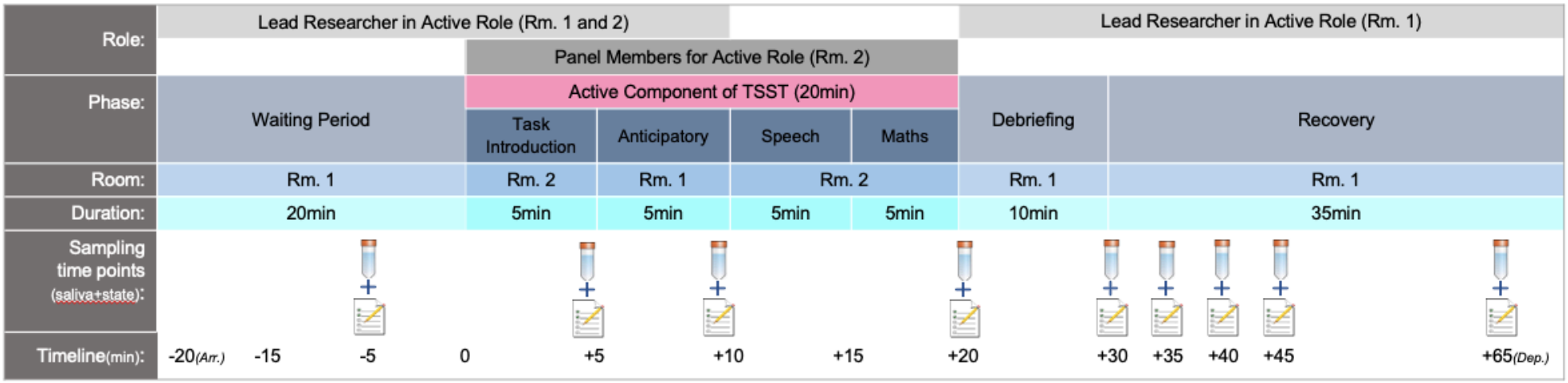


Figure 5.1. An illustration of the various phases of the TSST protocol. The top row (Researcher Roles) depicts the presence of each researcher for the separate Phases and Rooms of the TSST. The second row (Phase) depicts the separate phases involved in the TSST from beginning to end. Below the Phases, the Room row depicts where each of these phases takes place, i.e., Room 1 (Rm.1) being the waiting room, and Room 2 (Rm.2) being the TSST room. The Duration row includes the Duration, and depicts, in minutes, the length of each of the separate phases of the TSST. The red box in the duration row indicates the active components of the TSST and below this the TSST timeline is included. The TSST timeline is as per the original protocol (where time point 0 min indicates the onset of the active TSST, i.e., task instruction), with the participant arrival occurring at -20 min to TSST onset. As per the suggestions of this manuscript, the departure occurs at +65 min. Lastly, below the recommended TSST timeline, the figure includes the suggested timing of 9 sampling time points for cortisol and state psychological assessments, structured around the expected peak cortisol response.

2. General information and set-up

2.1 Testing time

- To occur in the ***afternoon***. For example, testing session to start at or after 2pm, with most common start times occurring between 2-4pm. Please ensure start time is kept consistent across all participants.
- For female participants, testing ideally to occur during the ***mid-luteal phase*** of their menstrual cycle, i.e., in the 14 days after the start of ovulation and prior to following menstrual period (between 14 – 28 days since the first day of the participants last menstruation).

2.2 Personnel

- A minimum of ***3 personnel*** is required:
  1 x Lead Experimenter to oversee and execute the overall protocol.
  1 x Active Panel member to lead the panel and deliver the instructions to participant.
  1 x Passive Panel member to complete the panel composition.
- The panel members to be mixed gender, e.g. 1 female + 1 male.
- The gender of the Active Panel member should be recorded down (i.e., whether cross gender or gender matched to the participant). Recording of this detail will allow for exploration of whether the gender of the Active Panel member influences stress response seen in the participant.

2.3 Equipment and Set-up Checklist

For more details please refer to the section '*Step-by-Step Protocol*'.

2.3.1 Waiting Room

This is Room 1 (Rm 1)

- ✓ Table and chair (see illustration below).
- ✓ Glucose drink
- ✓ Glass of water (100ml; for rinsing mouth following glucose drink; standardized amount for all participants)
- ✓ Magazines/reading material (of neutral content).
- ✓ Digital timer – for Lead Experimenter to follow.
- ✓ Participant documentation (e.g., information documentation and consent form).
- ✓ Physiological monitoring equipment (e.g., heart rate) ~ study specific.

2.3.2 Interview Room

This is Room 2 (Rm 2)

- ✓ Table/s and chairs (x 2) (see illustration below).
- ✓ Video camera and tripod (to face participant; camera should make a sound or have a red/coloured light visible to participant to indicate recording when turned on).
- ✓ Lab coats (x 3) for all personnel.
- ✓ Digital timers (x 2) (for Active Panel member with 5-min count down set; for other panel members with normal time of the day).
- ✓ Red cross on floor indicating participant's standing position.
- ✓ Arithmetic (maths) sequential number table (Appendix A).
- ✓ Observation sheets (Appendix B) for Passive Panel members.
- ✓ Scripts (instructions, stop protocol, panel scripts)(Appendix C, D, E).

Waiting Room

Interview Room

(3 person mixed gender panel presented above; 2 person minimum required)

2.3.3 Physiological monitoring equipment

- ✓ This is study specific and can include measuring heart rate and/or skin conductance (etc.), using a variety of equipment.

2.3.4 Brief state psychological measure

- ✓ Pre- and post-stress mood assessment, e.g., visual analogue scale (mood) and State-Trait Anxiety Inventory (state anxiety).
- ✓ Repeated assessments of mood during each saliva collection (sampling time point), e.g., visual analogue scale questions (see Appendix F).

2.3.5 Saliva collection

- ✓ Vials and collection aids (Passive drool) and/or salivettes (absorbent method)- each clearly labelled (ID and sample number)
- ✓ Gloves
- ✓ Labels
- ✓ Tissues
- ✓ Hand wash/sanitiser

2.4 Time keeping

- The Lead Experimenter is responsible for the timing of the overall protocol from time point -20 (Waiting Period) to completion at +65 (Participant Departure). It is recommended the Lead Experimenter carry a digital timer or watch throughout the protocol. There will be some minor variability in the overall 85-minute protocol timing to account for time variations such as transfer of participant from Rm 1 to Rm 2, sample collection and for scripts and instructions. While there may be some variability in the overall protocol, it is imperative the particular phases of the protocol are timed accurately and as instructed (e.g., 5-minute Speech phase; 5-minute Math phase).
- The Active panel member is responsible for timing the active stress phases (e.g., 5-minute Speech phase; 5-minute Math phase).
- Four digital timers are recommended; one to be carried by the Lead Experimenter, a second to remain on the table in front of the Active Panel member (Rm 2), a third for the Passive Panel member to follow (Rm 2), and lastly, a timer to track the overall timing of the protocol (to be left in Rm 1 and on display during Recovery so the participant may see the time remaining).

2.5 Saliva (cortisol) sampling

- It is very important to adhere to the time points for the designated cortisol samples across the 85-minute protocol (the manual depicts a 9-sample protocol; final sample number determined by individual projects – though time points to be standardized across entirety of testing).
- Lead Experimenter records exact time saliva samples collected.
- Ensure saliva sample tubes are carefully pre-labelled (e.g. date).
- All saliva sample collections are done in Rm 1.
- Saliva collection methods can vary (e.g., Passive drool, salivette, oral swabs). The *Passive drool method* is the most effective method and more ideal if interested in multiple analytes. For example, you could collect a sample using the Passive drool at the very start of the study to give you a baseline sample from which cortisol and other analytes can be examined. Then, the *salivettes (absorbent) method* may be used for a specific analyte, e.g. cortisol, as it is considered quicker and therefore more ideal for the repeated sampling points required for the TSST.
- Salivettes require 1 – 2 minutes to collect adequate volume. Researchers should be mindful under stress participants may experience dry mouth. Researchers should ensure the Salivette is "wet" enough and permit extra time to collect adequate sample volume if required. Consulting with the participant at the time of sampling is a useful way to determine if the mouth is dry.

Passive drool method	Salivette (absorbent) method
	

2.6 Personnel roles

- To maximise the cortisol stress response in participants, it is important that the Lead Experimenter, Active Panel member and Passive Panel member follow the prescribed scripts and guidelines for each individual role.
- Only the Lead experimenter and Active Panel member are to address the participant throughout the protocol. The remaining Passive Panel member(s) are to be courteous; any questions are to be directed to the Lead experimenter or Active Panel member.
- It is crucial that the panel members retain a neutral demeanour throughout the entire protocol until debriefing. The panel members should maintain eye contact with the participant throughout and offer their undivided attention. It is very important that the panel members do not take it upon themselves to be negative toward the participant with aggression or intentional meanness or alternatively to try and put the participant at ease with smiling or encouragement. Panel members should be courteous to the participant, but the emphasis should be on a strictly neutral demeanour for all panel members. To keep it natural, panel members are allowed to look down to their notes or record a note. The latter is especially useful if the panel member feels like he/she will break from their neutral demeanour (e.g., because participant is telling a joke).
- All personnel are to wear a lab coat throughout the entire protocol, i.e., until debriefing is completed.
- Rehearsal of the protocol is recommended prior to testing, including video recording of the panel for feedback.

3. Step-by-Step Protocol

3.1 Participant arrival and welcome @ -20 min

Upon participant arrival (approximately 20 minutes prior to the beginning of the TSST), the Lead Experimenter is to welcome the participant outside of the experiment rooms and obtain informed consent. The Lead Experimenter then takes the participant to Rm 1 (Waiting room), which serves as the waiting room and the room where speech preparation, debriefing and recovery will occur. Once the participant enters Rm 1, **Time Point -15** begins.

The Lead Experimenter starts the digital timer that will track the overall protocol.

3.2 Glucose drink @ -15 min

Upon entry to Rm 1, participant consumes a prepared glucose drink consisting of **200ml of sugar water** (30g pure dextrose powder dissolved in 200ml water). The amount and product used should be kept consistent across all participants. Immediately following the drink, participants get **100ml of still water** to rinse the mouth and are allowed to swallow this water. After this, participants are to refrain from drinking any additional water for the remainder of the protocol (note: this may be difficult for some participants who experience dry mouth because of the stress).

3.3 Waiting period @ -15 to 0 min

Following the glucose drink, the participant remains in Rm 1 to rest for 15 minutes prior to beginning the active stress component. Participants are not to complete extensive (e.g., cognitive) assessments during this period. Neutral reading material may be provided.
If heart rate is being monitored (optional), the application of any physiological recording should be applied (e.g., heart rate monitors) at the start of this period.

The Lead Experimenter reads the following script to the participant:

"This is the waiting period for your task today. You are to wait here comfortably for 15 minutes. I will be entering and exiting the room during the waiting period to complete some required tasks. Whenever I am not completing these I will leave the room.

If Heart Rate is recorded, the following script insert allows for a baseline standing HR to be recorded pre-completion of the TSST active components (also completed while standing

****I will return in 10 minutes to ask you to stand up for the remaining 10 minutes of this waiting period so that a standing baseline heart rate can be recorded.****

Continue with (regardless of HR insert):

Also, during the final 5 minutes of the waiting period, the first of your saliva samples will be collected and you will be asked to complete a brief questionnaire about your mood. You will complete this saliva collection and mood assessment 9 times throughout today's lab session."

3.3.1 Physiological assessment, e.g., heart rate

The inclusion of heart rate monitoring can involve two levels: a) continuous monitoring of the entire testing session, and b) monitoring of the 10-minute acute stress component in Rm 2. The equipment used will be study specific and dependent on equipment available in the laboratory.

3.3.2 Standing baseline heart rate @ -10 mins

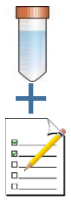
As the participant will complete the 10 minute active stress component while standing, a sample of their standing baseline heart rate is required. 10 minutes prior to entering Rm 2 for their task introduction, the participant is asked to stand and remain doing so until the completion of the TSST. The HR monitor applied in Rm 1 will record the participant's baseline standing heart rate prior to the stress task.

3.3.3 Pre-stress mood/state assessment @ -5 mins

Just prior to the end of the participant's Waiting Period and their first saliva collection (time-point -5mins) the participant is to complete a pre-TSST psychological state assessment, e.g., using the State-Trait Anxiety Inventory (STAI).

3.3.4 Saliva collection and mood assessment throughout protocol

Following completion of the pre-TSST STAI, the first cortisol sample is collected, along with a **brief psychological (mood) assessment,** e.g., 5 visual analogue scale questions (see Appendix F).



- ***1st Saliva collection & mood assessment (@ -5mins).***

3.3.5 Background questions (to ask during Waiting Period)

Cortisol Collection Questions (Record Answers):

Please ask the following questions to participant:

- ☐ At what time today did you first see daylight?
- ☐ When was your last drink? (not including the Glucose Drink)
- ☐ What was the drink?
- ☐ When did you last consume food?
- ☐ What was the food?
- ☐ When did you last exercise vigorously?

3.4 Task introductory period (pre-stress) @ 0 to +5 min

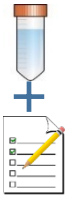
At time point 0, the Lead Experimenter collects the participant from Rm 1 and takes them to Rm 2.

The Lead experimenter tracks the 5-minute time allowance for the task introduction upon entry to Rm 2.

Upon entry to Rm 2, the 2 panel members are all sitting behind the long table with their lab coats on. A cross (X) is marked on the floor in the middle of the room. The Lead Experimenter instructs the participant to stand on the X and face the panel. Next to the panel, and directed towards the participant, is a video camera on a tripod.

To begin the Pre-Stress Task introductory period the Lead Experimenter is to read the following script to the participant:

"You will now begin the speech preparation portion of the task. In a moment, you will return to the waiting room where you will prepare a 5-minute speech. You are to take on the role of a job applicant who has been invited for a personal interview with the company's staff managers [the Lead experimenter to point towards the panel members]. **You are to convince these managers that you are the perfect applicant for the vacant position. These managers are specially trained to monitor nonverbal behaviour. Your speech will also be videotaped** [the Lead experimenter to point towards the video camera] **so that a video analysis of your voice frequency and performance may be conducted. You will find a notepad and pen in the waiting room to assist in your speech preparation. You may not bring these notes or cues with you when you return to the room. You will now have 5 minutes to prepare."**



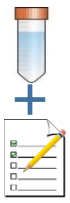
The Lead experimenter returns participant to Rm1 and collects cortisol sample number 2.

- ***2nd Saliva collection & mood assessment (@ +5mins)***.

3.5 Anticipatory stress period (speech preparation) @ +5 to +10 min

Following the collection of sample 2, the Lead Experimenter begins a 5 minute timer and leaves the room.

NOTE: should the participant ask any number of clarification questions once returning to Room 1, the lead experimenter should respond only where absolutely necessary and with a simple non-directive response, such as "**That is Unspecified**".



The participant is left alone in Rm 1 to prepare for their speech. When the timer is complete, the Lead Experimenter re-enters Rm 1 to collect sample 3. Once sample 3 has been collected the Lead Experimenter returns the participant to Rm 2.

- ***3rd Saliva collection & mood assessment (@ +10mins).***

NOTE: the 5 minute Task Introduction time allowance typically does not exceed more than 2 minutes. The additional time allows for moving between rooms, participant sampling, or clarification of participant questions (see above note).

3.6 Speech period (active stress) @ +10 to +15 min

Upon entry to Rm 2, the Lead Experimenter asks the participant to stand on the cross (X) on the floor.

The Active Panel Member has set the panel timer for two blocks of 5 minutes.

Prior to leaving Rm 2, the Lead Experimenter starts the required recording tasks. For example:

- ☐ Start the video recording equipment and begin recording
- ☐ Start any other recording equipment, e.g. Heart rate.

Once completed, the Lead Experimenter then leaves the room.

To begin the Speech period, the Active Panel Member is to read the following script to the participant:

"Welcome to your interview. We would like you to deliver your speech for the next 5 minutes. We will inform you when your time is complete. You may begin"

The Active Panel Member starts the 5-minute countdown on the timer.

If the participant stops their speech prior to the 5 minutes completion, the Active Panel Member is to respond in a standardized way:

- ☐ On the first instance of a pause, the Active Panel Member should first wait 20 seconds.
- ☐ If the participant is still silent at the end of the 20 seconds, the Active Panel Member should tell the participant:

"You still have some time left. Please continue"

- ☐ If the participant should pause again, the Active Panel Member should again wait 20 seconds.
- ☐ If the participant is still silent at the end of the 20 seconds, the Active Panel Member is to ask any one of the questions listed below. Aim to choose a question that is appropriate, i.e., that the participant has not already addressed in their speech. For example, if participant has spoken about their strengths, then avoid asking about this here. Repeat this procedure for any subsequent pauses (waiting 20 seconds each time prior to asking a question) until the 5 minute speech period is complete. These questions are in the script (see Appendix E):

"Why do you think you would be better suited for this job than the other applicants?"
"What qualities do you look for in your colleagues?"
"What qualities can you contribute to our company?"
"Do you prefer to work in a team or own your own?"
"What are some of your strengths?"
"What are some of your weaknesses?"

When the 5 minutes are up, the Active Panel Member should stop the timer and tell the participant:

"You may stop. You have reached the end of the speech portion of your task. You will now complete a 5-minute arithmetic task"

NOTE: If the participant directs questions to the panel during the speech component of the task, the active panel member should respond with "**You still have some time left, please continue**".

3.7 Arithmetic period (active stress) @ +15 to +20 min

Immediately following the speech, the Active Panel Member should then instruct the participant:

"Starting at one thousand and twenty-two, you are to serially subtract the number 13 as fast and as accurately as possible. You are to say your answers aloud. If your answer is incorrect, we will say "Stop, 1022". At each error, you are to start over, beginning again at one thousand and twenty-two and counting backwards in blocks of 13. This portion of the task will continue for 5 minutes. Your time begins now."

The Active Panel Member starts the 5-minute countdown on the timer.

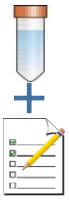
The Active Panel Member should follow along using the **Math Number Sequence Table** (see Appendix A).

NOTE: in the rare event that the participant reaches 0, please say the following to the participant **"Thank you. Please begin again at 1022 for the remainder of your time".**

When the 5 minutes are up, the Active Panel Member should stop the timer and tell the participant:

"Thank you, you may stop."

NOTE: If the participant does not comprehend or hear the instructions for the arithmetic task, the active panel member may repeat the full instructions for the task once.



At the end of the arithmetic period, the panel members should remain quiet, neutral and seated. The Lead Experimenter returns to Rm 2 to collect the participant and return the participant to Rm 1. Immediately upon entry to Rm 1, the Lead Experimenter collects the next saliva sample.

- ***4th Saliva collection & mood assessment (@ +20mins)***.

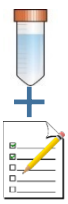
3.8 Debriefing period @ +20 to +30 min

Immediately following the saliva collection, the Lead Experimenter begins a 10 minute timer and the participant is to be debriefed.

The Lead Experimenter should debrief the participant as follows:

"You have just completed a task called the Trier Social Stress Test. This test is specifically designed to elicit psychological stress inside a lab environment. The speech and maths tasks you were asked to complete today were intentionally difficult and do not in any way reflect upon your aptitude or ability. The panel members were not evaluating or scoring you at all today and no video analysis will be conducted either. These measures were all prop elements designed to induce stress. We will not be comparing your performance to any other participants. What we were actually measuring today was the hormone cortisol which we collected through the saliva samples we took today. This is a natural occurring stress hormone, and the testing today allows us to get a picture of what happens in your body under stress. We apologise for not being completely honest about everything throughout the testing session, but we required the test to feel real or it would not be as stressful. You have done a really good job today and we really appreciate your effort and participation. Do you feel okay about everything? [allow participant to answer] **Are there any questions you would like to ask us?** [allow participant to ask questions and answer them as needed]**"**

- ☐ The Lead Experimenter is then to welcome the panel members into Rm 1 and introduce them to the participant. The panel members should remove their lab coats prior to meeting the participant in the debriefing.
- ☐ At the completion of the debriefing (time point +30), when the 10-minute timer ends, the Lead Experimenter collects the next saliva sample.



- ***5th Saliva collection & mood assessment (@ +30mins)***.

3.8.1 Post-stress state anxiety/mood assessment @ +30 mins

Just prior to starting the Recovery Period, the participant is to complete a post-TSST psychological assessment (e.g., STAI). As soon as participant has completed the post-TSST STAI, the participant may begin their Recovery Period.

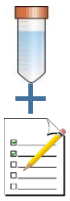
3.9 Recovery period @ +30 to +65 min

Following collection of the 5th saliva sample, the Lead Experimenter begins a 35-minute timer.

The Lead Experimenter should read the following script to the participant:

"You will now begin your recovery period. You will wait comfortably in this room for 35 minutes. I will enter in and out of the room at different time points to collect more saliva samples from you. Once the 35 minutes is complete and your final sample is collected, you will be finished for today."

The Lead Experimenter leaves Rm 1 and returns at the time points listed below for saliva collection. The timer runs continuously through this period until completion.



- ***6th Saliva collection & mood assessment (@ +35mins)***
- ***7th Saliva collection & mood assessment (@ +40mins)***
- ***8th Saliva collection & mood assessment (@ +45mins)***
- ***9th Saliva collection & mood assessment (@ +65mins)***

3.10 Participant departure @ +65 min

When the recovery timer ends (at time point +65) the participant may prepare to depart. Ensure prior to this time that the participant has no further questions and check the following:

- ☐ Check participant's wellbeing post completion of the lab session and answer any questions.
- ☐ Remove all equipment from participant.
- ☐ Check completion of all samples and questionnaires.
- ☐ Ensure samples are accurately labelled (ideally done prior to testing session).

Frequently Asked Questions

What time of day should the TSST be conducted? The TSST should be conducted within a time period that factors in the diurnal pattern of cortisol secretion (e.g., avoiding the CAR response period and periods of higher cortisol concentration at the beginning of the day/post-awakening) and avoids pre-meal slumps in cortisol stress response and post-meal increases in cortisol concentration. Typically, an afternoon scheduling of the TSST accounts for these factors (e.g. between 2pm – 5pm). Other start times (e.g., late morning) may still be considered as a feasible alternative, particularly for studies utilizing the TSST for purposes other than cortisol stimulation. Importantly, the timing should be kept consistent within a single experiment.

How long should the waiting and preparation time be? A waiting period of 15 minutes, with a 5-minute speech preparation period (anticipatory stress phase).

How long is the entire protocol? Protocol length may be reduced to 85 minutes total in order for reduction of participant burden and capture of peak cortisol stress response levels. Should capture of return to basal diurnal levels of cortisol be desired for a specific research study, the length of recovery phase of TSST protocol is to be increased in duration from 35 minutes to 60 minutes.

Can participants complete assessments prior to commencing the TSST? If assessments are utilized pre-TSST, these should be administered towards the end of the acclimation (waiting) period or alternatively immediately following the task introduction prior to participant commencing the anticipatory (speech preparation) phase.

What are the Panel arrangements? A two-member panel to be used composed of one male and one female, with the gender match of the active panel member to be recorded, according to the participant (e.g. active panel member gender matched or cross-matched).

How do the panel members behave during the TSST? All panel members are instructed to remain neutral (but not negative) toward the participant across the active component of the TSST and retain neutral facial expressions across the entire protocol.

Do the researchers need to dress a certain way? All panel members are to wear professional clothes appropriate to the environment. Recommended Lab coats are worn for consistency and to adhere to professional dress standards easily.

Can the participant be familiar to the panel members? Panel to be unknown to the participant.

Any requirements for testing Females compared to Males? All females should ideally complete the TSST during the same phase of their menstrual cycle and without contraceptives.

How many saliva samples are collected, and when? The number of samples to be collected is dependent upon the specific research question

for each study and the affordability of sampling. The proposed 9 collections in the current protocol allows for the capture of the full cortisol response profile. Alternatively, as few as 2 samples may suffice for some research questions (e.g., a manipulation check in a cognitive study).

How are saliva samples collected? Salivettes are practical and relatively easier to use for multiple cortisol collections but limited in volume and may be supplemented with passive drool sampling, such as pre- and post TSST.

Is a state psychological measure collected across the protocol? Some subjective assessment of the participant's experience during the TSST should be collected.

Is there a Glucose drink? Addition of glucose loading to a standardized TSST protocol via a Glucose drink, administered to all participants at the beginning of the waiting period for the TSST protocol.

Is there a Debriefing? Debriefing is a crucial part of the TSST and should contain a detailed exchange of details between the participant and lead researcher, aided by the panel members also being part, to ensure a thorough recovery process.

When to use the Stop Protocol? It has hard to determine when to stop the protocol because of distress. A general rule of thumb could be whenever it is felt necessary. Identifying the difference between *distress* and *significant distress* should be explained to all panel members during their induction and training to the TSST. It is up to the individual research lab to explore this and decide on terms and signs to look for. Panel members should be observing for and noting the presence of symptoms of panic such as: increased/trouble breathing, participant appears to struggle with chest pain or pressure, appearance of light-headedness or dizziness, appearance shakiness, excessive sweating, sudden flushing of skin, and crying. Panel members should then be informed about how to decide if they feel that these symptoms have presented in a manner that is too distressing for the participant. A useful resource to assist with this decision is the use of *a colour-coded system* for the panel members (see Appendix D).

What if the participant reaches 0 in the arithmetic task? If the participant reaches 0 during the 5-minute arithmetic task, the Active Panel Member is to say to the participant:

"Thank you. Please begin again, starting at 1,022 and counting backwards in blocks of 13 for the remainder of your time"

What about Heart rate collection? Other physiological markers of stress may be added to the TSST protocol, such as heart rate.

Best equipment for recording heart rate? Chest strap – fits tightly and least affected by arm movements that are common during speech delivery. Other options include wireless electrodes, wristband/watch etc.

Appendix A

Arithmetic Guide for Active Panel Member:

Math Period Sequential Number Table

Start Point: 1022	762	502	242
1009	749	489	229
996	736	476	216
983	723	463	203
970	710	450	190
957	697	437	177
944	684	424	164
931	671	411	151
918	658	398	138
905	645	385	125
892	632	372	112
879	619	359	99
866	606	346	86
853	593	333	73
840	580	320	60
827	567	307	47
814	554	294	34
801	541	281	21
788	528	268	8
775	515	255	-

Note: If the participant reaches 0 during the 5 minute arithmetic task, the Active Panel Member is to say to the participant:

“Thank you. Please begin again, starting at 1,022 and counting backwards in blocks of 13 for the remainder of your time”

Appendix B

Participant Observation Sheet (for panel members):

PARTICIPANT ID: DATE:

Speech Portion

Actual Start Time: _____

Time	Observation Note

Math Portion

Actual Start Time: _____

Time	Observation Note

Total Errors Made (Arithmetic):

Appendix C

Participant Instruction Sign (on panel table to face *participant* with Stop Protocol on reverse)**:**

Please stand on the **RED** cross

- During your task – please keep your

MOVEMENT TO A MINIMUM.

- Please try to limit

SWINGING, ANIMATING OR MOVING

your ARMS during the task.

- The Panel will

PROVIDE YOU WITH INSTRUCTIONS

and answer questions

ONLY WHEN APPROPRIATE.

Appendix D

A. Stop Protocol (optional) (on panel table to face *panel members*)**:**

STOP PROTOCOL

The TSST task is **meant to be a stressful experience** for the participant and is **designed to elicit psychological stress. We do not** however, want to allow the participant to reach a point of significant distress (panel members to be informed of what qualifies as significant distress prior to commencing any TSST panel).

Should the participant reach a point throughout the task where they become significantly distressed the **panel members should indicate to the other panel members they would like to discontinue. A collective decision by the panel should then be made and the TSST task discontinued.**

It is very important to ensure no harm comes to the participant. **Significant distress results in stopping the protocol.**

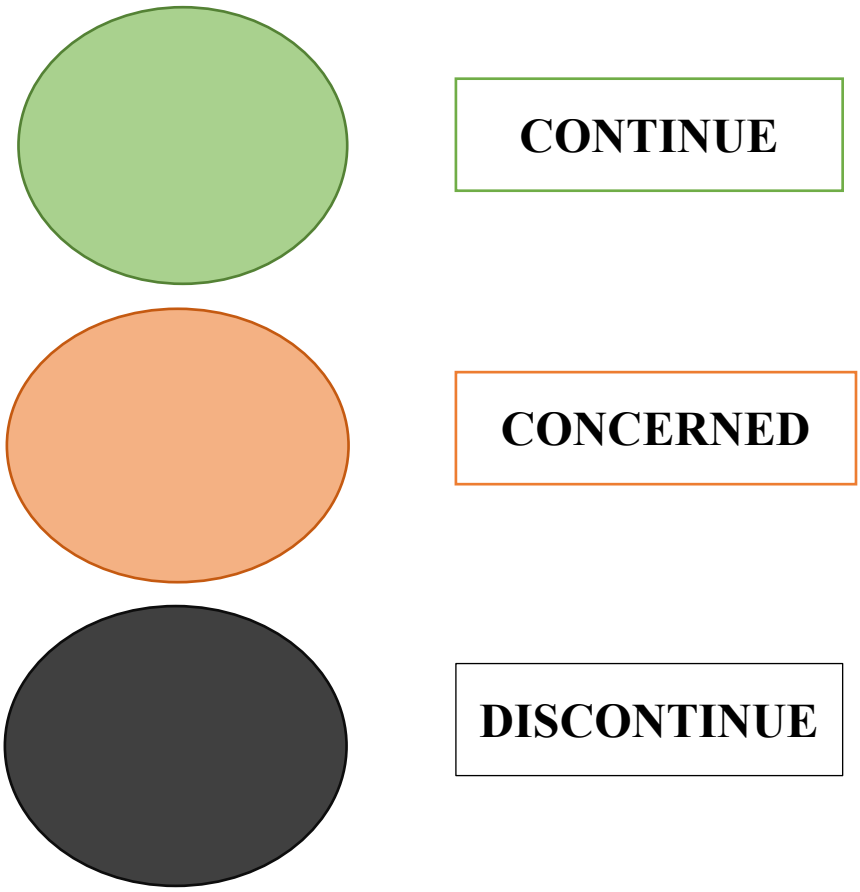
Please note that the protocol will induce discomfort and stress in the participant. **Do not stop the protocol if the distress is not significant.**

Please confer with your panel to collectively decide whether to continue or to stop the protocol.

B. Colour-coded system for panel consensus (optional)

To assist with determining whether to continue or stop an active protocol, a **colour coded system** can be employed by the panel. This system avoids panel members having to verbally converse or avert their attention from the participant during an active protocol. Each panel member should begin the TSST protocol with 3 colored circles overturned in front of them. One Green circle indicates that the panel member is happy to "continue" as normal (always begin the protocol with the panel members green circles colour facing upward). One Orange circle indicates "concern" whereby if the specific panel member has overturned the orange colored circle, they are indicating concern as to whether the panel should continue. One Black circle is used to indicate that the panel member who has overturned the black circle believes the panel should "discontinue" the protocol. The panel members should remain observant of the other panel member's circles and make a decision with their own colored circles. Should the panel members collectively overturn their black circles, the protocol should then be discontinued.

It should also be clearly discussed between the researchers how the protocol should be discontinued, who is responsible for initiating this and how the lead research will respond following discontinuation.



Appendix E

Lead Experimenter and Panel Member Scripts

A. Lead Experimenter Script

Waiting Period	"This is the waiting period for your task today. You are to wait here comfortably for 15 minutes. I will be entering and exiting the room during the waiting period to complete some required tasks. Whenever I am not completing these I will leave the room. **I will return in 10 minutes to ask you to stand up for the remaining 10 minutes of this waiting period so that a standing baseline heart rate can be recorded.** Also, during the final 5 minutes of the waiting period, the first of your saliva samples will be collected and you will be asked to complete a brief questionnaire about your mood. You will complete this saliva collection and mood assessment 9 times throughout the today's testing session."
Task Instruction	"You will now begin the speech preparation portion of the task. In a moment, you will return to the waiting room where you will prepare a 5-minute speech. You are to take on the role of a job applicant who has been invited for a personal interview with the company's staff managers [the Lead experimenter to point towards the panel members]. You are to convince these managers that you are the perfect applicant for the vacant position. These managers are specially trained to monitor nonverbal behaviour. Your speech will also be videotaped [the Lead experimenter to point towards the video camera] so that a video analysis of your voice frequency and performance may be conducted. You will find a notepad and pen in the waiting room to assist in your speech preparation. You may not bring these notes or cues with you when you return to the room. You will now have 5 minutes to prepare."
Debrief	"You have just completed a task called the Trier Social Stress Test. This test is specifically designed to elicit psychological stress inside a lab environment. The speech and maths tasks you were asked to complete today were intentionally difficult and do not in any way reflect upon your aptitude or ability. The panel members were not

	evaluating or scoring you at all today and no video analysis will be conducted either. These measures were all prop elements designed to induce stress. We will not be comparing your performance to any other participants. What we were actually measuring today was the hormone cortisol which we collected through the saliva samples we took today. This is a natural occurring stress hormone, and the testing today allows us to get a picture of what happens in your body under stress. We apologise for not being completely honest about everything throughout the testing session, but we required the test to feel real or it would not be as stressful. You have done a really good job today and we really appreciate your effort and participation. Do you feel okay about everything? [allow participant to answer] Are there any questions you would like to ask us? [allow participant to ask questions and answer them as needed]"
Recovery	"You will now begin your recovery period. You will wait comfortably in this room for 35 minutes. I will enter in and out of the room at different time points to collect more saliva samples from you. Once the 35 minutes is complete and your final sample is collected, you will be finished for today."

B. Active Panel member Script

Speech Period	"Welcome to your interview. We would like you to deliver your speech for the next 5 minutes. We will inform you when your time is complete. You may begin" **1st PAUSE:** **First WAIT 20 SECONDS…. IF still paused say: "**You still have some time left. Please continue" **2nd PAUSE:** **First WAIT 20 SECONDS…. IF still paused ask: (choose appropriate question to participants speech)** "Why do you think you would be better suited for this job than the other applicants?" "What qualities do you look for in your colleagues?" "What qualities can you contribute to our company?" "Do you prefer to work in a team or own your own?"

	"What are some of your strengths?" "What are some of your weaknesses?" **END of 5 Minutes:** "You may stop. You have reached the end of the speech portion of your task. You will now complete a 5-minute arithmetic task" NOTE: 2 If the participant asks questions of the panel during the speech portion of the task, the active panel member may respond with "you still have some time left, please continue"
Math Period	"Starting at one thousand and twenty-two, you are to serially subtract the number 13 as fast and as accurately as possible. You are to say your answers aloud. If your answer is incorrect, we will say "Stop, 1022". At each error, you are to start over, beginning again at one thousand and twenty-two and counting backwards in blocks of 13. This portion of the task will continue for 5 minutes. Your time begins now." "Thank you. You may stop." NOTE: • If participant reaches 0 say: "Please begin again at 1,022 until your time is finished" • If the participant does not comprehend or hear the instructions initially, the active panel member may repeat the full instructions once.

C. Passive Panel member Script

Throughout:	• Maintain a neutral demeanour throughout entire protocol – this is imperative to the study. • Be courteous! Maintain interest and eye contact throughout the participant's task. • Direct any questioning from participant back toward the Lead Experimenter or the Active Panel member. • Do not address the participant directly. • You may take notes throughout the participant's task to play the role further. • NO aggression • No encouragement (Nods, Smiles)

Appendix F

Brief Visual Analogue Scale (example questions)

Please rate the way you feel in terms of the dimensions given below. Regard the line as representing the full range of each dimension (0 being the lowest value, 100 being the highest value). Rate your feelings as they are at the moment.

0 10 20 30 40 50 60 70 80 90 100

Happy

Sad

Tired

Anxious

Withdrawn

Appendix G

Luteal Phase Questionnaire for Female Participants

For females:

Menstrual Cycle :

Are you on the contraceptive pill?
❒ Yes. Name of Pill: _____
❒ No

If No, do you have a regular cycle?
❒ Yes. Average cycle length: _____ days
❒ No

Current cycle phase:
How many days has it been since the first day of your last menstruation?
_____ days

Cycle phase estimation (For researcher to complete_
❒ menstrual phase
❒ follicular phase (roughly days 1-14)
❒ luteal phase (roughly days 14-28)

CHAPTER 6. Empirical Study 1 – Acute Social Stress in Social Anxiety Disorder: Physiologically the Same, Subjectively Different

Abstract

Background: Social anxiety disorder (SAD) is one of the most prevalent anxiety disorders with debilitating impairments in social functioning and daily living. Yet, there is a dearth of research examining the physiological and subjective experience of social stress in SAD. Currently, there is evidence to suggest discordance between physiological and subjective reactivity in those with SAD, however there are inconsistencies in the research. We employed an acute social stress task in the laboratory to examine the physiological and subjective stress response in those with SAD compared to healthy controls.

Methods: Participants were 40 individuals with a primary diagnosis of social anxiety disorder (50% female) and 41 age- , sex-, and education-matched healthy controls who completed the Trier Social Stress Test. Salivary cortisol concentration and self-ratings on visual analogue mood scales (anxiety, sadness, tiredness, withdrawal and happiness) collected at nine timepoints across the protocol served as physiological and subjective measures of acute stress, respectively. Preliminary analyses checked for possible cortisol non-responders compared to cortisol responders across all participants. Analyses are therefore reported by group (SAD vs. healthy controls) and responder status (non-responders vs. responders). Cortisol data were analysed in terms of two area under the curve measures (total growth and time dependent reactivity), calculated across the nine timepoints.

Results: Across 81 total participants, 17 participants (21%) were classified as cortisol non-responders, with proportionate numbers of non-responders in the SAD and healthy control groups. After non-responders were removed, there were no group differences on salivary cortisol levels at baseline or over the course of the stress protocol. Cortisol responders and non-responders showed no differences at baseline, however over the course of the stress protocol, responders had significantly higher cortisol reactivity compared to non-responders. Subjectively, the SAD group reported significantly higher levels of anxiety, sadness, tiredness, and withdrawal, and lower levels of happiness compared to the control group at baseline and in the overall magnitude of response over the stress protocol. Moreover, the SAD group also experienced greater overall intensity in anxiety and sadness, over the stress protocol compared to the healthy control group. When statistically accounting for temporal dynamics between the cortisol and anxiety responses to acute stress, we found evidence for a moderate positive association that did not differ between groups.

Conclusions: This study lends support to the those with SAD having similar physiological but largely different subjective responses to acute stress, relative to healthy controls. In contrast to previous studies, we found evidence for concordance in subjective and physiological stress reactivity when we accounted for the time-dependent differences in the two response systems. This research suggests the examination of stress in SAD should observe the stress response broadly across a number of distinct stress systems as dysfunction in one stress response system may not generalise to others (e.g. subjective stress but not adrenocortical dysfunction in SAD). Future research should examine in more detail the nature of the adrenocortical functioning in SAD during social stress.

Keywords: cortisol, HPA axis, humans, public speaking, mental arithmetic, TSST

1. Introduction

Social anxiety disorder (SAD) is a common and debilitating mental health disorder characterised by excessive fear and anxiety for potential judgement, scrutiny and negative evaluation by others. This fear and anxiety is significantly disruptive to the individual's life (Heimberg & Magee, 2014). Individuals with SAD are at greater risk of school drop-out and under-employment, as well as lower workplace productivity, socioeconomic status, well-being, interpersonal relationships and quality of life (Patel et al., 2002). In Australia, SAD is the second most prevalent anxiety disorder, with a 12-month prevalence rate of 1% and a lifetime prevalence rate of over 8% (Australian Bureau of Statistics, 2008). Given the debilitating nature and significant impacts to everyday life, along with the prevalence of the disorder, understanding how those with SAD react to social situations is critical to improving treatment strategies. However, because SAD is known to involve biased appraisals and avoidance of social situations, studying how individuals with SAD respond to naturally occurring social stressors is very challenging. A useful alternative involves experimentally inducing social stress in the laboratory using standardised social stress tasks.

A growing body of evidence suggests that people with SAD report greater anxiety and fear, but differ little, if at all, in physiological responding to experimentally induced social stressors compared to healthy controls (Jamieson, Nock, & Mendes, 2013; Klumbies et al., 2014; Mauss, Wilhelm, & Gross, 2003). However, previous studies have not statistically tested the association between the subjective and physiological experience of acute stress in SAD, despite reports of discordance between the response systems (Jamieson et al., 2013; Klumbies et al., 2014). Investigating the temporal association between the subjective and physiological experience of SAD may contribute to better understanding of the disorder and improved treatment methods and management of the experience of those

with SAD. Investigating the temporal association between the subjective and physiological experience of SAD may contribute to better understanding of the disorder and improved treatment methods and management of the experience of those with SAD. There are a number of cognitive models of SAD that propose the maintenance of the disorder is largely cognitive (see Clark & Wells, 1995; Heimberg, Brozovich, & Rapee, 2014; Hofmann, 2007; Rapee & Heimberg, 1997) and suggest that when faced with a socially threatening situation, individuals with SAD shift their attention inward – engaging with increased self-monitoring and a number of maladaptive cognitive strategies to manage their experience (Hirsch, Clark, Mathews, & Williams, 2003). If evidence supports that the dysfunction in SAD is largely cognitive, with the physiological salivary cortisol response to social stress being comparative between SAD and healthy controls, then the development of new, and improvement of existing, intervention strategies can use a more targeted approach. Identifying and fostering a better understanding of the key underlying mechanisms that contribute to the functional impairment and maintenance of SAD is paramount to developing improved treatments for the disorder.

In those with SAD, subjective reports consistently denote increased experiences of anxiety and fear, along with negative affect and cognitions, when presented with social stressors (Jamieson et al., 2013; Klumbies et al., 2014; Mauss et al., 2003). In contrast, research into the physiological (e.g., cortisol) stress response in SAD has delivered varying results. Some studies report that, when compared to healthy controls, there is no difference in the salivary cortisol reactivity of those with SAD in response to an acute social stressor (see Klumbies et al., 2014; Krämer et al., 2012; Martel et al., 1999). Others have found that individuals with SAD reporting significantly higher cortisol reactivity to acute social stressors relative to the controls

(see Condren et al., 2002; Furlan, Demartinis, Schweizer, Rickels, & Lucki, 2001; Roelofs et al., 2009; van West, Claes, Sulon, & Deboutte, 2008).

A number of reasons potentially account for inconsistencies in these results, including but not limited to (i) differences in the social stress tasks employed (e.g., modified versions of the Trier Social Stress Test; Kirschbaum et al., 1993) that limits comparison between studies; (ii) differences in the characteristics of the sample groups, such as age (e.g., lack of generalisability between child and adolescent populations to adults; Allen et al., 2014; van West et al., 2008), gender (e.g., sex differences in stress response are known; Allen et al., 2014) and presence of comorbidities (e.g., with or without depression); (iii) the presence of within-group differences (e.g., cortisol responders and non-responders; those who exhibit a minimum prerequisite salivary cortisol response and those who do not; Klumbies et al., 2014; Miller, Plessow, Kirschbaum, & Stalder, 2013); and (iv) differences in the physiological outcome of interest (e.g., salivary cortisol, heart rate, salivary alpha amylase) and how these are analysed (e.g., slope, area under the curve).

A further limitation of current studies appears to be the lack of direct statistical examination of the association between the subjective and physiological response to acute stress in SAD. Whether there is a functional association between our physiological and subjective experiential response systems has in general received very little attention to date (Sommerfeldt, Schaefer, Brauer, Ryff, & Davidson, 2019). For decades, it has been assumed that there is coherence in our affective, physiological and behavioural responses to stress that serve an adaptive function (Ekman, 1992; Lazarus, 1991; Levenson, 1994; Sommerfeldt et al., 2019). It is now widely understood however, that while interrelated, there is a lack of coherence across the different stress response systems with each representing a number of independent and varying components rather than a single, synchronised stress system

(Sommerfeldt et al., 2019). In a review, Campbell and Ehlert (2012) examined whether subjective stress response corresponds to the physiological stress response in healthy controls during completion of the Trier Social Stress Test (TSST; Kirschbaum et al., 1993). Of the 30 studies examined, only 27% (n = 8) of the studies demonstrated significant correlations between cortisol responses and subjective emotional stress variables in healthy controls (Campbell & Ehlert, 2012). No associations were found for changes in anxiety and salivary cortisol responses (Cohen et al., 2000; Ditzen et al., 2007; Therrien et al., 2010) and self-reported anxiety was not found to predict peak salivary cortisol response or changes in salivary cortisol from pre-stress to peak (see Fox, Cahill, & Zougkou, 2010; Jones, Rollman, & Brooke, 1997). In contrast, Rimmele et al. (2007) demonstrated that differences in stress reported pre- and post-stressor were significantly correlated with salivary cortisol area under the curve (AUC; as a summary measure).

With physiological and psychological stress responses theoretically representing the same construct (i.e. stress), it is predicted a strong association between the two responses would be observed, e.g. high subjective and physiological stress covariance (Schlotz et al., 2008). It is unclear why there is low coherence between these subjecitve and physiological stress reponse systems. It may be that the calculated associations do not account for the different temporal dynamics of the systems, i.e. the slow acting endocrine response lagging behind the more immediate psychological responses. Further, these studies predominantly assessed subjective reports pre- and post-stressor, thereby potentially missing the highly sensitive, time-contingent changing emotional states which precede the slow-acting HPA axis response (Schlotz et al., 2008). Schlotz et al (2008) employed repeated real-time assessment of subjective emotional states during different stress episodes (not pre- and post-assessment) and further accounted for the expected time-lag between the two

stress responses (psychological and physiological) during analyses. These authors found a strong positive relationship between state anxiety and cortisol response was found in healthy controls, with changes in anxiety representing same-direction changes in salivary cortisol.

Whether assessment is between-person or within-person is also highly important as these assessments of concordance are interpreted differently. Between-person assessment of concordance refers to individuals who respond highly on one measure (e.g. anxiety) also responding with high levels on another measure (e.g. cortisol). Within-person concordance, however, refers to the correlation between two measures (e.g. anxiety and cortisol) over time, within a single individual, such that as salivary cortisol increases so do subjective reports of anxiety. Within-person concordance reflects how the two measure move together across time and therefore requires repeated assessments. We argue that it is more likely for there to be within-person concordance between subjective anxiety and salivary cortisol in response to an acute stressor rather than between-person concordance (Mauss, Levenson, McCarter, Wilhelm, & Gross, 2005).

A final point to consider, raised by Sommerfeldt et al. (2019), is that concordance observed between physiological and subjective stress responding may relate to a number of influencing factors, such as participant sex (Avero & Calvo, 1999), menstrual cycle (Nicolson et al., 1997), attachment style (Ditzen et al., 2008) and variance in external and internal problems (Hastings et al., 2009). The average concordance observed may be low due to individual differences in concordance, with some individuals potentially even demonstrating negative covariance between the different components involved in the stress response. Individual variations such as the above need to be considered in regard to the role they play.

To date, studies on SAD have only examined the relationship between physiological and subjective stress response independently. Despite correlational evidence of a lack of coherence (Campbell & Ehlert, 2012) and Klumbies and colleagues (2014) reporting discordance findings in their study, direct statistical examination of concordance or discordance are lacking. Moreover, current evidence on physiological and subjective stress response in SAD has not accounted for certain modulating factors of the stress response; in this study, it included sex, attachment, personality, self-esteem and perceived childhood maltreatment.

1.1 The Current Study

In the current study, the goal was to examine the subjective and physiological acute stress response in those with SAD. This study aimed to replicate findings showing heightened subjective stress responses, but not physiological (cortisol) stress response differences in those with SAD compared to healthy controls (Jamieson et al., 2013; Klumbies et al., 2014). We also aimed to extend these findings by statistically testing for within-person concordance in the subjective and physiological stress responses between the SAD and control groups.

Social stress was experimentally induced using the TSST (Kirschbaum et al., 1993) using a recently published introductory guide from this thesis (See Chapter 5). In brief, the TSST is one of the most widely used research tools for the induction of moderate acute psychobiological stress in experimental research (Allen et al., 2017). Physiologically, a strong salivary cortisol response following the TSST is used as a marker of an acute stress response. Subjectively, the TSST has been shown to lead to increased self-reported stress, anxiety, negative mood/affect and wakefulness (Allen et al., 2014; Kirschbaum et al., 1999). The protocol focused on salivary cortisol sampling for measurement of the physiological acute stress response as practical cost-

effective biomarker involving minimal participant burden. Five visual analogue mood scales (VAMS) were included to assess the self-reported subjective experience of acute stress. The current study also accounted for potentially confounding differences in characteristics of the sample groups by closely matching the two groups on factors such as age, sex and level of education while also incorporating a strict exclusion criterion for the presence of comorbidities. Furthermore, this study distinguished between cortisol responder (R) and non-responder (NR) subgroups and examined whether these subgroups differed in their subjective acute stress response or according to group status (healthy controls vs. SAD).

1.2 Aims and Hypotheses

Our specific aims were three-fold. First, the study aimed to compare groups (SAD vs. controls) on salivary cortisol levels across the stress protocol (9 time points). Second, groups were compared on subjective experiences across the stress protocol. Lastly, to test for concordance, the study aimed to statistically examine within-person associations between the subjective (i.e. anxiety) and physiological (cortisol) stress response in SAD as compared to controls. We also examined the influence of other variables as potentially modulating factors on the physiological acute stress response (aim 1), such as childhood mistreatment, personality traits and the population sub-groups of cortisol responders vs. non-responders.

For aim one, we expected no significant difference in salivary cortisol concentration at baseline and across the protocol in response to acute social stress in SAD when compared to controls. This prediction is based on recent evidence from studies that have also utilised the TSST (Klumbies et al., 2014). We also explore salivary cortisol in relation to a number of potentially modulating factors, including sex, childhood mistreatment, personality, attachment and severity of social anxiety.

For aim two, we hypothesised higher levels of anxiety, sadness, tiredness and withdrawal, and lower levels of happiness in SAD at baseline, when compared to controls. We predicted those with SAD would demonstrate higher overall growth in anxiety, sadness, and withdrawal and a greater overall decrease in happiness in response to the stress protocol, and further demonstrate greater reactivity in anxiety in response to the acute stress protocol. These hypotheses were based on the consistent evidence of higher baseline levels of negative affect and lower reports of positive affect in those with SAD (Cohen et al., 2017; Gross & Jazaieri, 2014), and increased negative affect and decreased positive affect in response to acute social stress (Jamieson et al., 2013; Klumbies et al., 2014).

For aim three, when accounting for the different temporal dynamics of the stress response systems (Schlotz et al., 2008) and examining within-person concordance, previous literature in control populations has been inconsistent (see Campbell & Ehlert, 2012), while existing concordance studies specific to SAD have not examined the relationship directly (e.g. Klumbies et al., 2014). Functional transformations, such as a phase shift, can be used in statistical analyses to account for the different temporal dynamics of different variables. A phase shift involves shifting a function horizontally along the x-axis (in the case of this study, time) from the observed position. When accounting for a phase shift to accommodate the different temporal nature of the subjective and physiological responses in healthy controls, Schlotz et al (2008) found strong coherence between the subjective and physiological stress response. We predicted that there would be stronger concordance between subjective and physiological acute stress response in the control group than in the SAD group.

2. Methods

2.1 Participants

Forty SAD participants (20 females) and 41 healthy control individuals (20 females) comparable in age, sex and total years of education participated in this study (see Table 6.1 for participant characteristics). Participants were recruited using online advertisements and flyers distributed around inner Melbourne. General inclusion criteria required participants to be between the ages of 18–55 years of age, be non-smoking, no substance abuse, be medication free and not currently engaged in psychotherapeutic intervention, and proficient in English language. Due to the impact of menstruation cycle phases on cortisol levels in response to psychosocial stressors (Kirschbaum et al., 1999) all female participants were tested in the luteal phase of their cycle.

SAD participants were required to have a current or suspected diagnosis of SAD. The Mini International Neuropsychiatric Interview (MINI 7.0.2 Full and MINI 7.0.2 Screen; English version for the DSM–5; Sheehan et al., 1998) was conducted to confirm SAD diagnosis and record the presence, *if any*, of other comorbid mental disorders. Participants for the clinical group were included in the study if they met the diagnostic criteria for SAD as their principal diagnosis (determined through the use of the MINI 7.0.2 and interview with the participant if required) and if they reached a score ≥36 on the Social Interaction Anxiety Scale (SIAS; Mattick & Clarke, 1998) and ≥30 on the Leibowitz Social Anxiety Scale (LSAS; Liebowitz, 1987). SAD is commonly associated with comorbid disorders, such as generalised anxiety and depression. Participants with a history of post-traumatic stress disorder, a psychotic disorder (e.g. bipolar disorder or schizophrenia), a current major depressive episode, an intellectual disability or neurodevelopmental disorder were excluded from participation.

Table 6.1

Group Demographic Characteristics and Cortisol Responder Status of Social Anxiety Disorder (SAD) and Healthy Control (HC) Participants

N = 81		SAD (*n* = 40)	HC (*n* = 41)	Test	*p*	*df*
Gender (Female)	*n (%)*	20 (50.0)	20 (48.8)	$\chi^2 = 0.01$	.913	1
Age (Years)	*M (SD)*	28.42 (7.90)	25.75 (6.36)	$t = 1.68$	.098	79
Education (Total Years)	*M (SD)*	16.18 (2.25)	16.57 (2.09)	$t = 0.83$	.412	79
Education (Level Completed)						
Primary & Secondary School	*n (%)*	9 (22.5)	14 (34.1)			
TAFE, Certificate, Diploma	*n (%)*	5 (12.5)	3 (7.3)			
Tertiary Degree	*n (%)*	21 (52.5)	18 (43.9)			
Postgraduate Tertiary Degree	*n (%)*	5 (12.5)	6 (14.6)			
Hormonal Contraceptives	*n (%)*	7 (17.5)	12 (29.3)	$\chi^2 = 1.56$	.211	1
Contraceptive Pill	*n (%)*	4 (10.0)	11 (26.8)			
Inter-Uterine-Device/Implant	*n (%)*	3 (7.5)	1 (2.4)			
None	*n (%)*	13 (32.5)	8 (19.5)			
Cortisol Non-Responders	*n (%)*	10 (25.0)	7 (17.1)	$\chi^2 = 0.77$	.381	1

Note: *M(SD)* = mean (standard deviation), *n* (%) = number (percentage), *df* = degrees of freedom, SAD = social anxiety disorder, HC = healthy control, χ^2 = chi-square test, *t*= t-test of independence, *p* = *p*-value significant at $p < 0.05$ level, * $p < .05$. ** $p < .01$. ***$p < .001$

Where there was the presence of comorbid disorders, only the following were to be accepted, under the condition they were secondary to the SAD diagnosis: generalised anxiety disorder (GAD) and symptoms of low mood (though not a current depressive episode). Any possible presence of a comorbid disorder identified on the MINI *screen* questionnaire (e.g., GAD) was followed up with the corresponding *full* MINI module (e.g. Module N; GAD). No participant in this study was reported to meet criteria for a comorbid disorder in the current participant group.

While so many 'pure' cases of SAD are rare in clinical practice, the additional eligibility criteria (e.g., non-smoking, no substance abuse) for the general screening eliminated a large number of participants who were likely meeting comorbid diagnoses but were either a) engaged in psychotherapeutic intervention (treatment seeking) OR were managing their experience with medication, thus excluded from participation. To further characterise SAD, the total score of the SIAS (Mattick & Clarke, 1998), the LSAS (Liebowitz, 1987) and the State-Trait Anxiety Inventory (STAI; Spielberger et al, 1983) trait anxiety items were utilised. Further clinical assessment of depressive, anxiety and stress symptoms included the Depression Anxiety Stress Scale (DASS-21; Lovibond & Lovibond, 1995). Assessment of self-esteem was done via the Rosenberg Self-Esteem Scale (RSE; Rosenberg, 1965). See Table 6.2 for clinical characteristics.

Further, the presence of any other clinically significant medical (e.g., diabetes, cancer), neurodevelopmental disorder (e.g., attention deficit/hyperactivity disorder) or neurological condition excluded individuals from participation. Control participants were to have no current or suspected diagnosis for any mental illness (using MINI 7.0.2). Written informed consent was provided by all participants prior to their inclusion in the study and ethical approval for the conduct of the study was approved by the Australian Catholic University Human Research Ethics Committee. Participants were remunerated a maximum $150AUD on a pro-rata basis dependent upon their overall participation in the larger study components (this laboratory visit being embedded within a larger protocol involving EMA).

Table 6.2

Group Clinical Characteristics of Social Anxiety Disorder (SAD) and Healthy Control (HC) Participants

N = 81		SAD (*n* = 40)		HC (*n* = 41)		Test	*p*	*df*
Social Anxiety Severity								
SIAS	*M (SD)*	57.00	(9.26)	16.08	(10.30)	*t* = 18.69	< .001***	78
LSAS	*M (SD)*	78.70	(19.05)	24.26	(16.25)	*t* = 13.21	< .001***	73
Psychological Variables								
STAI (Trait)	*M (SD)*	55.10	(7.58)	36.05	(6.93)	*t* = 11.67	< .001***	77
DASS-21 Depression	*M (SD)*	17.00	(8.61)	3.56	(3.62)	*t* = 9.20	< .001***	79
DASS-21 Anxiety	*M (SD)*	18.85	(8.18)	4.15	(5.34)	*t* = 9.61	< .001***	79
DASS-21 Stress	*M (SD)*	24.70	(8.16)	8.29	(5.96)	*t* = 10.35	< .001***	79
RSE	*M (SD)*	14.15	(4.32)	22.49	(4.34)	*t* = 8.67	< .001***	79
Attachment - ASQ								
Confidence	*M (SD)*	25.58	(6.36)	35.46	(5.41)	*t* = 7.35	< .001***	75
Relationship as Secondary	*M (SD)*	20.32	(5.13)	16.03	(5.11)	*t* = 3.65	< .001***	74
Need for Approval	*M (SD)*	30.05	(4.22)	20.74	(6.15)	*t* = 7.72	< .001***	75
Discomfort with Closeness	*M (SD)*	43.00	(7.69)	32.13	(7.87)	*t* = 6.17	< .001***	76
Relationship Preoccupation	*M (SD)*	33.67	(6.66)	24.28	(6.77)	*t* = 6.17	< .001***	76
Personality - BFI								
Openness to Experiences	*M (SD)*	3.82	(0.58)	3.63	(0.62)	*t* = 1.37	.174	78
Conscientiousness	*M (SD)*	3.22	(0.60)	3.76	(0.66)	*t* = 3.83	< .001***	78
Extraversion	*M (SD)*	2.35	(0.66)	3.38	(0.72)	*t* = 6.68	< .001***	78
Agreeableness	*M (SD)*	3.48	(0.65)	3.88	(0.55)	*t* = 2.92	.005**	78
Neuroticism	*M (SD)*	3.94	(0.59)	2.61	(0.72)	*t* = 9.00	< .001***	78
Environmental - CTQ								
Emotional Abuse						*FE*	.002**	
None to Minimal	*n (%)*	17	(45.95)	33	(82.50)			
Low to Extreme	*n (%)*	20	(54.05)	7	(17.50)			
Physical Abuse						*FE*	.026*	
None to Minimal	*n (%)*	22	(57.90)	36	(87.80)			
Low to Extreme	*n (%)*	16	(42.10)	5	(12.20)			
Sexual Abuse						*FE*	.044*	
None to Minimal	*n (%)*	29	(74.35)	37	(92.50)			
Low to Extreme	*n (%)*	10	(25.65)	3	(7.50)			
Physical Neglect						*FE*	.181	
None to Minimal	*n (%)*	21	(53.85)	30	(73.17)			
Low to Extreme	*n (%)*	18	(46.15)	11	(26.83)			
Emotional Neglect						*FE*	< .001***	
None to Minimal	*n (%)*	11	(28.20)	30	(73.17)			
Low to Extreme	*n (%)*	28	(71.80)	11	(26.83)			
Minimisation Denial						*FE*	.043*	
None to Minimal	*n (%)*	33	(82.50)	26	(63.41)			
Low to Extreme	*n (%)*	7	(17.50)	15	(36.59)			

Notes: M(SD) = mean (standard deviation), *n (%)* = number (percentage), *df* = degrees of freedom, SAD = social anxiety disorder, HC = healthy control, SIAS = social interaction anxiety scale, LSAS = Leibowitz social anxiety scale, STAI = state trait anxiety inventory, DASS-21 = depression anxiety stress scale, RSE = Rosenberg self-esteem scale, ASQ = attachment style questionnaire, BFI = big five inventory, CTQ = childhood trauma questionnaire, *FE* = Fisher's exact test of independence, *t* = t-test, *p* = *p*-value significant at $p < 0.05$ level, * $p < .05$. ** $p < .01$. ***$p < .001$

2.2 Procedure

After phone screening and successful inclusion into the study, all participants commenced the larger study involving a social stress test (reported here) embedded within an 8-day EMA protocol and at-home saliva sampling (reported as Study 2 in Chapter 7). Participants completed two lab visits for the overall larger study, the first lab visit completed on Day 1 involving a number of clinical characterisation and trait characteristic questionnaires along with an introduction to the overall protocol and methods (i.e. phone software and at home saliva sampling kits). Participants returned to the lab on Day 5 of the 8-day testing cycle to complete the second lab visit and the social stress component. For the social stress test, participants completed the TSST (Kirschbaum et al., 1993) based on the recently updated introductory guidelines for how to conduct the TSST that consisted of an 85-min protocol (see Chapter 5).

Briefly, the TSST protocol included: a waiting period (20 min), an active TSST component including a task introduction (5 min), anticipatory period (5 min), speech component (5 min), and surprise arithmetic task (5 min), then a debriefing period (10 min) and recovery phase (35 min). See Figure 6.1 for sampling overview.

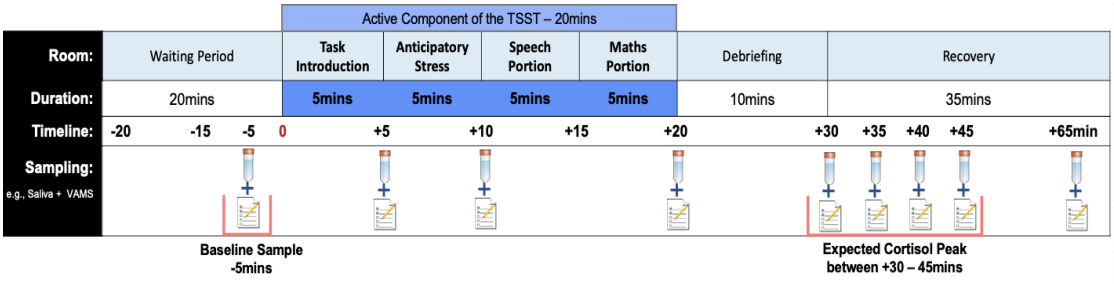


Figure 6.1. Various phases of the standardised TSST protocol. The top row (Phase) depicts the separate phases involved in the TSST from beginning to end. The Duration row includes the duration, and length of each phase of the TSST. The figure includes the timing of the 9 sampling time points for cortisol and state affect assessment, highlighting baseline sample and the expected peak cortisol response.

The active component of the TSST was completed in front of a panel of 3 independent mixed-gender "judges". Saliva samples for cortisol analyses and

subjective assessment of anxiety and affect via five VAMS were obtained at 9 time points (-5, +5, +10, +20, +30, +35, +40, +45, +65).

2.2.1 Physiological stress. Across the TSST, nine saliva samples were collected with Salivettes (Sarstedt) to assess salivary cortisol as a measure of HPA axis activity in response to acute stress. These were stored at -80 degree Celsius until analysed. Samples underwent one freeze thaw cycle. For analyses, saliva samples were thawed and analysed using commercially available kits (Salimetrics, USA) according to the manufacturer's instructions (Stratech Scientific APAC PTY Ltd). The Salimetrics cortisol assay kit is a competitive immunoassay specifically designed to measure salivary cortisol. Thawed samples were centrifuged at 1500 x *g* for 15 min to collect clear saliva and this saliva was used without further processing for all assays. All samples were brought to room temperature before adding to the assay wells and all samples were analysed in duplicate. Intra- and inter-assay coefficients of variation were at 4.3% and 4.6% respectively for salivary cortisol. Salivary cortisol correlates well with matched serum cortisol concentrations; $r = 0.91$, assay sensitivity equal to .003µg/dL. All analyses were within the set proficiency standard. Seven samples had insufficient volume for analysis across all participants. In-house methods of minimum sample recovery were tried however analysis was not able to be completed so they were noted as missing samples. Five of these samples were from one participant, who was excluded from further physiological analyses. Two remaining missing samples were replaced with statistical analyses (refer to statistical section). Final salivary cortisol analyses included 40 SAD and 40 healthy controls.

2.2.2 Subjective stress. A mood scale was administered at each of the nine sampling time points across the TSST to assess subjective changes in mood and anxiety. This shortened version of *the Visual Analogue Mood Scale* (VAMS; Bond & Lader, 1974) was designed for use specific to this study and included five items:

Happy, Sad, Tired, Anxious, and Withdrawn. For each scale, participants rated the way they felt at the time on a line representing the full range of each dimension (0 being the lowest value, 100 being the highest value; completed on an iPad); see Figure 6.2.

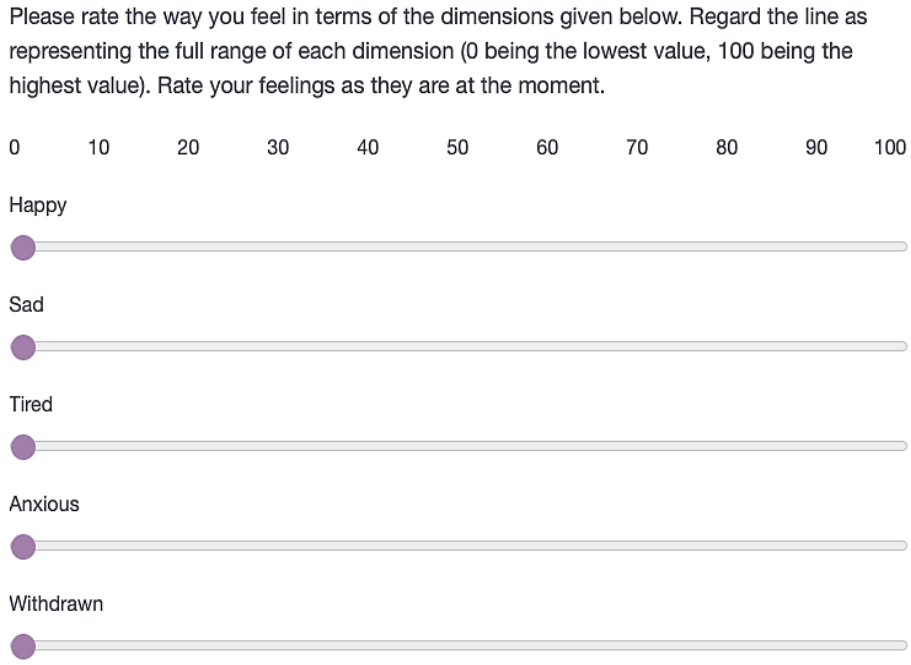


Figure 6.2. Brief VAMS measure designed to assess mood across acute stress task.

2.3 Statistical Analysis

Statistical Package for the Social Sciences (SPSS; IBM Corp, 2015), Stata (StataCorp, 2019) and JASP open-source statistical software (JASP Team, 2019) were used to perform all statistical analyses for this study. Data was first checked for missing values and outliers. Where variables were not normally distributed, non-parametric tests were used. Participants with ≤3 (out of the nine) salivary cortisol sample values missing had missing values replaced with the expectation maximisation algorithm in SPSS (n = 2). Missing values for the VAMS subscales were also replaced using the expectation maximisation algorithm (time points 1-4 and 9) in SPSS (n = 2). Participants with ≥4 salivary cortisol samples missing were excluded

from analyses (n = 1). Outliers with z-scores of more than +/-3 were excluded from any physiological statistical analyses, this resulted in 11 observations (from n = 3 participants) being excluded from further salivary cortisol analyses where appropriate. The significance level was $\alpha = 0.05$ for all analyses (two-tailed).

2.3.1 Responders and non-responders. Previous research has found the presence of a subgroup of people who do not respond with the expected salivary cortisol excretion in response to stress, known as cortisol non-responders (Miller et al., 2013). Prior to running the main analyses, salivary cortisol data were checked for the rate of responders (R) and non-responders (NR). The statistical model for classifying cortisol R and NR for this study was the threshold of baseline-to peak cortisol increases of 1nmol/l (.03 µg/dL) for samples analysed by the Salimetrics immunoassay method, as determined by Miller, Plessow, Kirschbaum and Stalder (2013). To calculate baseline-to-peak cortisol increase, each participant's baseline cortisol concentration (i.e., collected 5min prior to TSST-onset) was subtracted from their peak, defined as the highest salivary cortisol concentration from samples 6 to 8 (i.e., 35 to 45min post TSST-onset, during which the highest post-stressor cortisol response is typically shown). While we report on differences between R and NR, the main focus for this study were differences at the clinical group level (i.e. SAD vs. HC).

2.3.2 Baseline analyses. Baseline salivary cortisol and subjective measures (happy; sad; tired; anxious; withdrawn) were obtained from the very first sample (of the nine samples) collected; see Figure 6.1. To examine baseline predictions in Aim 1 and Aim 2, we first tested for baseline differences between groups on salivary cortisol and subjective ratings. Given that we predicted no group difference in salivary cortisol at baseline and conventional significance testing does not allow evidence for the null hypothesis, we used Bayesian hypothesis testing to obtain an estimate of the degree of

evidence for the null hypothesis (vs. the alternate hypothesis of group differences). The goal was to determine whether any significant differences in baseline salivary cortisol between SAD vs. HC and NR vs. R were present. Guidelines for conducting and reporting Bayesian analysis were used (van Doorn et al., 2019), specific to JASP open-source statistical software (JASP Team, 2019). A Bayesian Mann-Whitney U test allowed for us to express evidence for the null hypothesis, or the alternative, i.e. evidence for or against the presence of an effect (Rouder, Speckman, Sun, Morey, & Iverson, 2009). The null hypothesis postulates that there is no difference in baseline cortisol between the groups and therefore $H_0 : \delta = 0$

The Bayes factor is the likelihood of one particular hypothesis to the likelihood of another and indicates a measure of the strength of evidence in favour of one hypothesis compared to the other. The Bayes factor is written as BF_{10} and indicates evidence for H_1 (the alternate hypothesis).

2.3.3 Area under the curve (AUC) analyses. Area under the curve (*AUC*) was calculated using data from all nine time-points to quantify total cortisol and subjective responding across the TSST. Two formulas for calculation of AUC derived from the trapezoid formula include the 'Area under the curve with respect to ground' (AUC_g) and the 'Area under the curve with respect to increase' (AUC_i) (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). The AUC_g indicates whether any changes occurred over time (i.e., did the stress protocol have an overall effect/ the overall systemic output), while the magnitude of the AUC_i relates to the rate of change over time in the outcome (i.e., time-dependent reactivity).

To calculate the AUC_g, measurement values and the time distance between these measurements were required. For this data set, nine repeated measures were sampled with variable time between measures. The formula used to derive AUC_g:

$$AUC_g = \sum i \ = 1n - 1\left(m_{(i+1)} + m_i\right) \circ t_i 2$$

Where t_i denotes the time interval between each pair of successive measurements, m_i the individual measurement of salivary cortisol, and n the total number of measurement occasions. To calculate the measure of time dependent reactivity, the formula used to derive AUC_i:

$$AUC_i = \left(\sum_{i=1}^{n-1} \frac{\left(m_{(i+1)} + m_i\right) \circ t_i}{2}\right) - \left(m_1 \circ \sum_{i=1}^{n-1} t_i\right)$$

Where t_i denotes the time interval between successive measurements, m_i the individual measurement of salivary cortisol, and n the total number of measurement occasions. In addition to salivary cortisol, AUC was derived for subjective mood measures also.

2.3.4 Concordance between subjective and physiological stress.

Concordance between subjective and physiological responses during the TSST was estimated using within-person correlations. Two statistical approaches may be used to examine how (within-person) concordance is associated with an individual-difference variable (e.g. HC/SAD and R/NR). The first is a two-step approach, in which a within-person correlation coefficient is calculated separately for each participant in the first step, and subsequently these within-person correlation coefficients are correlated with the individual-differences variable in the second-step. The second approach involves linear mixed-effects modelling (LMEM) to examine whether the within-person effect of one stress response measure (e.g. subjective ratings) on the other stress response measure (e.g. cortisol) is moderated by an individual-differences variable (e.g. group status). The LMEM approach is statistically preferable (Hox, Moerbeek, & Van de Schoot, 2018), although can be less intuitive to interpret (Sommerfeldt et al., 2019). We therefore conducted both forms of analyses.

To account for the different temporal dynamics of the slow acting HPA axis (i.e., cortisol) response and the faster-acting subjective experience, our analyses included a phase shift (see Schlotz et al., 2008) to align the expected peak responses of the two systems. We note this is standard practice in research examining emotional concordance (see Mauss et al., 2005). We focus specifically on anxiety and cortisol responders, as our interest was in stress reactivity and whether there is concordance between subjective (i.e. anxiety) and physiological (i.e. cortisol) measures of the stress response. Subjective anxiety was expected to begin to increase between baseline (sample 1) and task introduction (sample 2) and to decline following task cessation and debriefing (between samples 4 to 5). Peak salivary cortisol was expected to occur between sample 5 to sample 8 (see justification in Chapter 5). We therefore applied a phase shift to align expected subjective peaks with expected cortisol peaks, shifting subjective sampling forward four time points, with correlations conducted on the time-lagged data. We include five time points in the phase shift analyses.

3. Results

3.1 Sample and Demographic Results

A total of 96 individuals were recruited for this study, with nine participants not commencing participation due to cancellation (n = 5), loss of contact (n = 2) and change of mind (n = 2), leaving 87 participants to commence participation. A further 6 participants did not complete their participation for various reasons (n = 2 refused the TSST, n = 1 due to illness, n = 1 left due to new work commitments, n = 1 displayed poor compliance and attendance, and n = 1 dropped-out). Therefore, this left a final sample of 81 participants comprising 40 SAD and 41 healthy controls for

statistical analysis. There were no statistically significant group differences in the demographic variables (sex, age, hormonal contraceptives, education; see Table 6.1).

3.2 Physiological Stress Response

3.2.1 Responders and non-responders. Using the threshold of baseline-to-peak cortisol increases of 1nmol/l (.036 µg/dL; Miller et al., 2013), 17 of the 81 participants who completed the protocol were classified as NR (21%). The proportion of NR did not differ between the SAD and healthy control groups (see Table 6.1).

3.2.2 Baseline measure. A Bayesian Mann-Whitney U test for groups difference (SAD vs. HC) in baseline cortisol concentration yielded a Bayes factor of $BF_{10} = .334$ (95% credible interval = -.605 to .226). This indicates that the data are approximately .334 times more likely to occur under the alternative hypothesis than under H_0 (the null hypothesis; see Figure 6.3a), reflecting on weak or *anecdotal* evidence for H_1 (Lee & Wagenmaker, 2013). Similarly, Bayesian Mann-Whitney U test comparing baseline cortisol concentration among R vs. NR yielded a Bayes factor of $BF_{10} = .282$ (95% credible interval = -.382 to .548), meaning that the data are approximately .282 times more likely to occur under H_1 than under H_0 (see Figure 6.3b). This result indicates *moderate* evidence for H_1 (Lee & Wagenmaker, 2013).

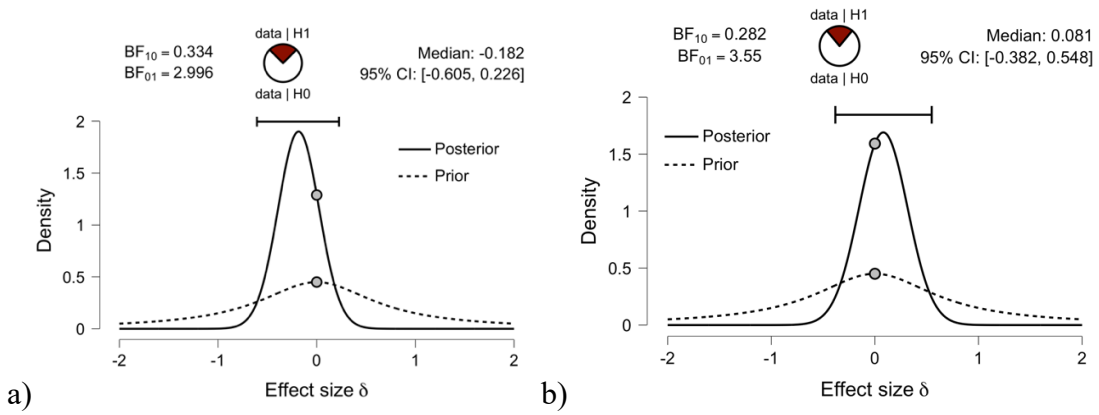


Figure 6.3. Illustration of Bayesian Mann-Whitney U baseline salivary cortisol comparisons for a) Social anxiety disorder vs. healthy controls and b) Responder vs. Non-responder.

No current option for a Bayesian Kruskal-Wallis comparison is available. A regression analysis was performed to compare baseline salivary cortisol between the four subgroups (SAD-R; SAD-NR; HC-R; HC-NR), with 1,0000 Markov-Chain Monte-Carlo runs and 2,500 burn-ins. Median (Q1 to Q3) baseline salivary cortisol for the subgroups and median differences (95% credible interval) between HC-NR (reference) and the other subgroups based on the regression analysis are listed in Table 6.3. Results indicate no baseline differences between the four subgroups.

Table 6.3

Median Salivary Cortisol Baseline for the Four Subgroups and Baseline Differences from Regression Analyses with 10000 Model Runs.

N = 81	Median Baseline (Q1 to Q3)	Median Baseline Differences	95% CrI
HC – NR (*n* = 6)	.231 (.138 to .291)	Ref	-
HC – R (*n* = 34)	.194 (.154 to .279)	-0.21	-1.08 to .62
SAD – NR (*n* = 8)	.296 (.177 to .432)	0.08	-.98 to 1.13
SAD – R (*n* = 32)	.221 (.169 to .339)	-0.15	-.93 to .86

Note. Median (Q1 to Q3) = median (interquartile range), HC-NR = healthy control cortisol non-responder, HC-R = healthy control cortisol responder, SAD-NR = social anxiety disorder cortisol non-responder, SAD-R = social anxiety disorder cortisol responder, 95% CrI = 95% credible interval, ref = reference

3.2.3 AUC. A Bayesian Mann-Whitney U test for group differences (SAD vs. HC) in salivary cortisol AUCi yielded a Bayes factor of BF_{10} = .247 (95% credible interval = -.347 to .481). This indicates the data are approximately .247 times more likely to occur under the H_1 than under H_0 (see Figure 6.4a), reflecting *moderate* evidence for H_1 (Lee & Wagenmaker, 2013). Similarly, a Bayesian Mann-Whitney U test comparing salivary cortisol AUCi among R vs. NR yielded a Bayes factors of

BF_{10} = 256.023 (95% credible interval = -1.577 to -.472), meaning that the data are approximately 256.02 times more likely to occur under H_1 than under H_0, reflecting *extreme* evidence for H_1 (difference between R and NR AUCi).

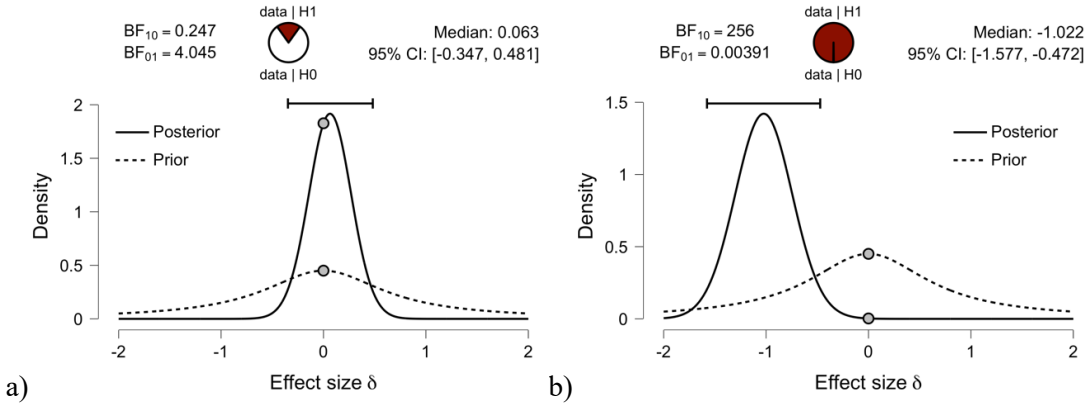


Figure 6.4. Illustration of Bayesian Mann-Whitney U AUCi comparisons for a) Social anxiety disorder vs. healthy controls and b) Responder vs. Non-responder.

A Bayesian Mann-Whitney U test for group differences (SAD vs. HC) in salivary cortisol AUCg yielded a Bayes factor of BF_{10} = .257 (95% credible interval = -.321 to .502). This indicates the data are approximately .257 times more likely to occur under the H_1 than under H_0 (see Figure 6.5a), reflecting *moderate* evidence for H_1 (Lee & Wagenmaker, 2013). Similarly, a Bayesian Mann-Whitney U test comparing salivary cortisol AUCg among R vs. NR yielded a Bayes factors of BF_{10} = 34.31 (95% credible interval = -1.319 to -0.277) meaning that the data are approximately 34.31 times more likely to occur under H_1 than under H_0 (see Figure 6.5b, reflecting *very strong* evidence for H_1 (difference between R and NR AUCg).

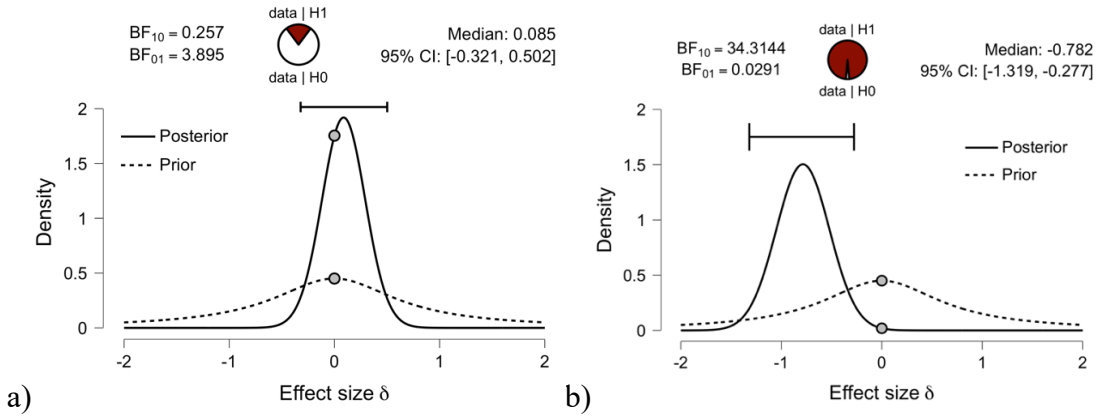


Figure 6.5. Illustration of Bayesian Mann-Whitney U AUCg comparisons for a) Social anxiety disorder vs. healthy controls and b) Responder vs. Non-responder.

Baseline salivary cortisol, AUC statistics and comparisons for group (SAD vs. HC) and responder status (R vs. NR) are presented in Table 6.4. Figure 6.6 depicts median salivary cortisol concentration across time for groups and responder status.

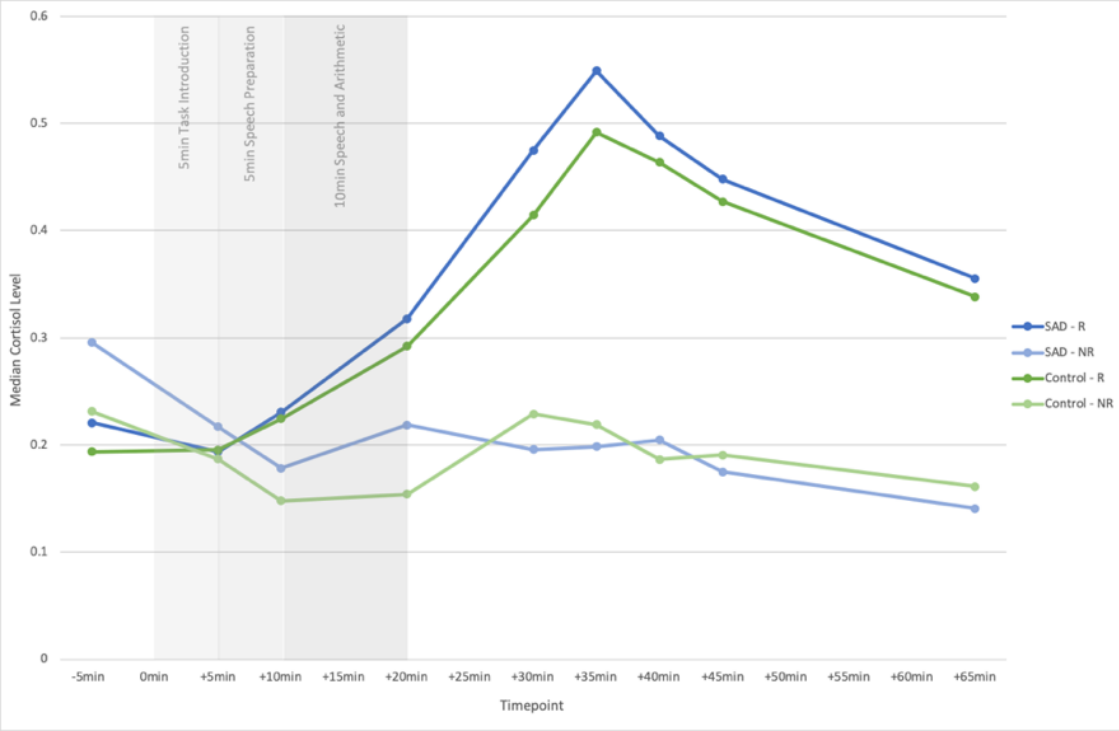


Figure 6.6. Median salivary cortisol concentration (ug/dL) across time for group and responder status (SAD-R; SAD-NR; HC-R; HC-NR).

Table 6.4

Baseline Salivary Cortisol and Repeated Measures Salivary Cortisol (ug/dL) Area Under the Curve (AUC) Median and Comparison for Group Status (SAD and HC) and Responder Status (R and NR)

N = 81		SAD (*n* = 40)	HC (*n* = 41)	Test
Baseline Salivary Cortisol (ug/dL)	Median (Q1 to Q3)	.242 (.166 to .375)	.194 (.151 to .284)	*W* = 949.50
Salivary Cortisol AUC_i	Median (Q1 to Q3)	6.70 (-.06 to 12.16)	7.11 (1.48 to 11.71)	*W* = 735.00
Salivary Cortisol AUC_g	Median (Q1 to Q3)	22.53 (16.24 to 29.85)	23.17 (17.66 to 31.89)	*W* = 726.00
		R (*n* = 64)	NR (*n* = 17)	Test
Baseline Salivary Cortisol (ug/dL)	Median (Q1 to Q3)	.209 (.160 to 0.305)	.244 (.156 to 0.417)	*W* = 483.50
Salivary Cortisol AUC_i	Median (Q1 to Q3)	8.58 (3.84 to 15.16)	-2.09 (-9.43 to -0.55)	*W* = 1028.00
Salivary Cortisol AUC_g	Median (Q1 to Q3)	24.62 (18.57 to 34.80)	16.24 (7.67 to 18.37)	*W* = 913.00

Note. Median (Q1 to Q3) = median (interquartile range), SAD = social anxiety disorder, HC = healthy control, R = cortisol responder, NR = cortisol non-responder, *W* = Bayesian Mann-Whitney U test statistic

3.2.4 Modulating factors. Mixed-effects regression was used to examine whether statistically modulating factors, including perceived childhood maltreatment, attachment style, self-esteem and personality had an effect on salivary cortisol in response to acute social stress. Results of the analyses remained similar when controlling for childhood maltreatment (i.e. CTQ factors), personality (i.e. BFI

factors), attachment style (i.e. ASQ factors) and self-esteem (i.e. RSE), despite differences in these factors at the group level (i.e. SAD and HC).

3.3 Subjective Stress Response

3.3.1 Baseline measure. A series of Mann-Whitney U tests revealed that the SAD group had significantly higher baseline measures of self-reported anxiety, tiredness, sadness and withdrawal, and significantly lower baseline measures of happiness when compared to the healthy control group; see Table 6.5. Responder and non-responders did not differ on baseline self-reported anxiety, happiness, sadness, tiredness or withdrawal; see Table 6.6.

Table 6.5

Comparison of Baseline Subjective Measures for Group (SAD and HC)

N = 81		SAD (*n* = 40)	HC (*n* = 41)	Test	*Z*	*p*	*r*
Anxiety	Median	67.50	15.00	*U* = 149.00	-6.34	< .001***	.70
	(Q1 to Q3)	(48.25 to 83.00)	(3.00 to 36.50)				
Happiness	Median	50.00	77.00	*U* = 230.00	-5.58	< .001***	.62
	(Q1 to Q3)	(21.50 to 63.50)	(67.00 to 84.00)				
Tiredness	Median	61.50	31.00	*U* = 527.00	-2.77	.006**	.31
	(Q1 to Q3)	(25.50 to 81.25)	(17.00 to 52.00)				
Sadness	Median	22.50	2.00	*U* = 252.50	-5.39	< .001***	.60
	(Q1 to Q3)	(8.00 to 50.75)	(.00 to 4.00)				
Withdrawal	Median	38.50	5.00	*U* = 260.00	-5.30	< .001***	.59
	(Q1 to Q3)	(22.00 to 58.00)	(1.00 to 17.00)				

Note. Median(Q1 to Q3) = median (interquartile range), SAD = social anxiety disorder, HC = healthy control, R = cortisol responder, NR = cortisol non-responder, *U* = Mann-Whitney U test for group comparison, *r* = effect size for group difference, *p* = *p*-value significant at *p* < 0.05 level, * *p* < .05. ** *p* < .01. ***p <.001

Table 6.6

Comparison of Baseline Subjective Measures for Responder Status (R and NR)

N = 81		R (*n* = 64)	NR (*n* = 17)	Test	*Z*	*p*	*r*
Anxiety	Median	46.50	40.00	*U* = 531.00	-0.15	.880	.01
	(Q1 to Q3)	(13.50 to 67.65)	(13.50 to 77.00)				
Happiness	Median	70.00	61.00	*U* = 447.00	-1.13	.260	.13
	(Q1 to Q3)	(50.00 to 80.75)	(26.00 to 77.50)				
Tiredness	Median	44.00	45.00	*U* = 487.50	-0.66	.512	.07
	(Q1 to Q3)	(20.00 to 63.00)	(21.00 to 78.50)				
Sadness	Median	6.00	6.00	*U* = 449.50	-1.10	.270	.12
	(Q1 to Q3)	(1.00 to 23.50)	(2.00 to 51.50)				
Withdrawal	Median	19.00	20.00	*U* = 532.50	-0.13	.894	.01
	(Q1 to Q3)	(3.00 to 40.00)	(2.50 to 43.50)				

Note. Median (Q1 to Q3) = median (interquartile range), SAD = social anxiety disorder, HC = healthy control, R = cortisol responder, NR = cortisol non-responder, *U* = Mann-Whitney U test for group comparison, *r* = effect size, *p* = *p*-value significant at $p < 0.05$ level, * $p < .05$. ** $p < .01$. ***$p < .001$

3.3.2 AUC. Bayesian Mann-Whitney U tests were used to test for group differences in AUC statistics (SAD vs. HC; R vs. NR). The SAD group had statistically significantly higher measures of AUC_g for the self-reported measures of anxiety, sadness, tiredness and withdrawal and statistically significantly lower AUC_g measures of happiness when compared to the control group; see Table 6.7. No associations were observed between responder status and AUC_g anxiety, happiness, sadness, tiredness or withdrawal. Participants in the SAD group had statistically significantly larger measures of AUC_i for the self-reported measures of anxiety and sadness, but not for happiness, tiredness or withdrawal; indicating greater time dependent reactivity in anxiety and sadness compared to healthy controls; see Table 6.7. For a detailed explanation of the influence of magnitude in AUC_i see Fekedulegn

et al (2007). No associations were observed between responder status and AUC_i for anxiety, happiness, sadness, tiredness or withdrawal.

Table 6.7

Comparison of Social Anxiety Disorder (SAD) and Healthy Control (HC) Subjective Measure AUC_g and AUC_i

N = 81		SAD (*n* = 40)	HC (*n* = 41)	Test	*z*	*p*	*r*
Anxiety AUC_g	Median (Q1 to Q3)	4078.75 (2695.00 to 4756.88)	1220.00 (747.50 to 2265.66)	*U* = 136.00	-6.46	< .001***	.72
Happiness AUC_g	Median (Q1 to Q3)	2620.00 (1601.88 to 3410.00)	4902.50 (4390.00 to 5557.50)	*U* = 208.00	-5.78	< .001***	.64
Tiredness AUC_g	Median (Q1 to Q3)	3746.25 (1835.00 to 5414.38)	1822.50 (1110.00 to 3390.00)	*U* = 475.00	-3.26	.001***	.36
Sadness AUC_g	Median (Q1 to Q3)	1393.75 (538.13 to 2551.25)	197.50 (13.75 to 687.50)	*U* = 253.50	-5.36	< .001***	.60
Withdrawal AUC_g	Median (Q1 to Q3)	2377.50 (1365.00 to 3931.25)	540.00 (93.75 to 1023.75)	*U* = 210.50	-5.76	< .001***	.64
Anxiety AUC_i	Median (Q1 to Q3)	-712.50 (-1321.25 to 184.38)	95.00 (-451.25 to 556.25)	*U* = 504.50	-2.98	.003**	.33
Happiness AUC_i	Median (Q1 to Q3)	-653.75 (-1201.41 to 314.38)	-385.00 (-1097.50 to -32.50)	*U* = 809.00	-0.10	.917	.01
Tiredness AUC_i	Median (Q1 to Q3)	-75.00 (-858.13 to 335.00)	-262.50 (-995.00 to 83.75)	*U* = 758.50	-0.58	.561	.06
Sadness AUC_i	Median (Q1 to Q3)	-331.25 (-800.00 to 96.88)	.00 (-28.75 to 137.50)	*U* = 526.50	-2.78	.006**	.31
Withdrawal AUC_i	Median (Q1 to Q3)	-410.94 (-945.62 to 721.25)	.00 (-227.50 to 80.00)	*U* = 681.00	-1.31	.189	.15

Note. Median (Q1 to Q3) = median (interquartile range), SAD = social anxiety disorder, HC = healthy control, R = cortisol responder, NR = cortisol non-responder, AUCg = area under curve with respect to ground, AUCi = area under curve with respect to increase, *U* = Mann-Whitney U test, *r* = effect size, *p* = *p*-value significant at $p < 0.05$ level, * $p < .05$. ** $p < .01$. ***$p < .001$

3.4 Discordance and Concordance in Physiological and Subjective Stress

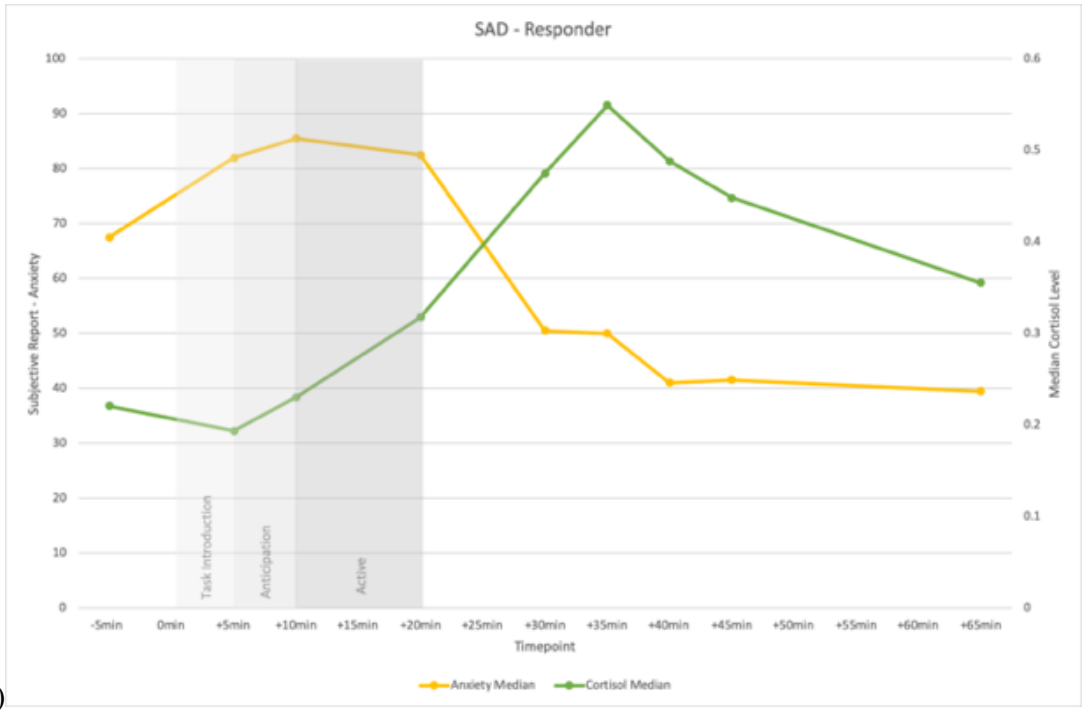
Given that cortisol NRs showed no change in cortisol concentration over time, they could not demonstrate within-person concordance among physiological (i.e., cortisol) and subjective stress responding. Thus, only cortisol responders (n = 30 SAD and n = 34 HC) were included in the following analyses testing for concordance among subjective (anxiety) and physiological (salivary cortisol) stress reactivity.

Using within person correlation coefficient analyses, we first plotted the median cortisol/anxiety association across time for SAD and healthy control responders separately (see Figure 6.7a and b). Visual inspection of the data showed a clear early subjective peak response relative to a delayed peak cortisol response, as expected. This, as well as knowledge of recommended time-window to capture the peak salivary cortisol stress response, i.e. between 30-45min post task onset (see Chapter 5), suggested a phase-shift of the subjective anxiety responses from time-point 1 (-5 min) to time-point 5 (30+ min; 10 min post-cessation of active TSST). For these analyses, only 5 time-points were analysed.

Following a phase-shift in the anxiety data (see Figure 6.8a and 6.8b), results showed that there was a moderate-to-strong positive cortisol/anxiety association for both the SAD and healthy control groups, Median (Q1 to Q3) of .405 (-.207 to .800) for SAD (n = 30) and .400 (.052 to .600) for healthy controls (n = 33). A Mann-Whitney U test revealed no statistically significant difference between the two groups, U = 486.50, z = -0.117, p = .907, r = .01. The findings suggest that there were coherent subjective and physiological responses across all participants when adjusting for the different temporal dynamics of subjective versus HPA-axis reactivity. To check the robustness of our within-person correlation analyses, we re-ran the above analyses using linear mixed-effects models (LMEM). In these models, we tested

whether the effect of anxiety on cortisol (modelled at level-1) was moderated by clinical group (SAD vs. HC; modelled at level-2) accounting for a phase shift. Results were similar to the two-step within-person correlation approach, reported above.

a)



b)

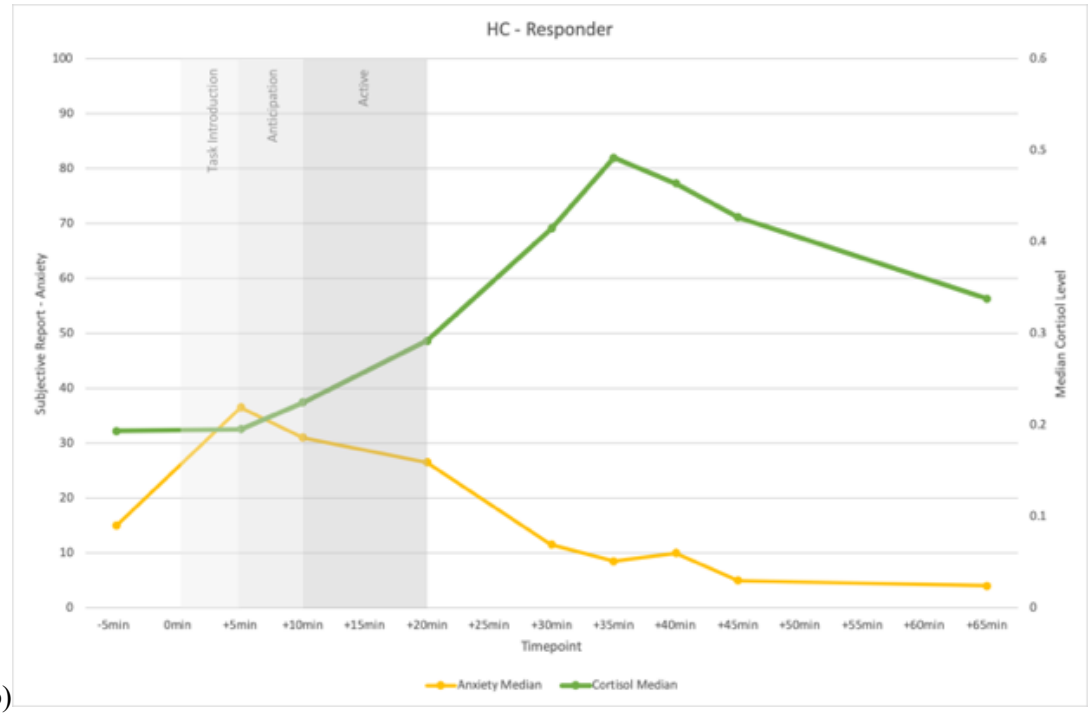
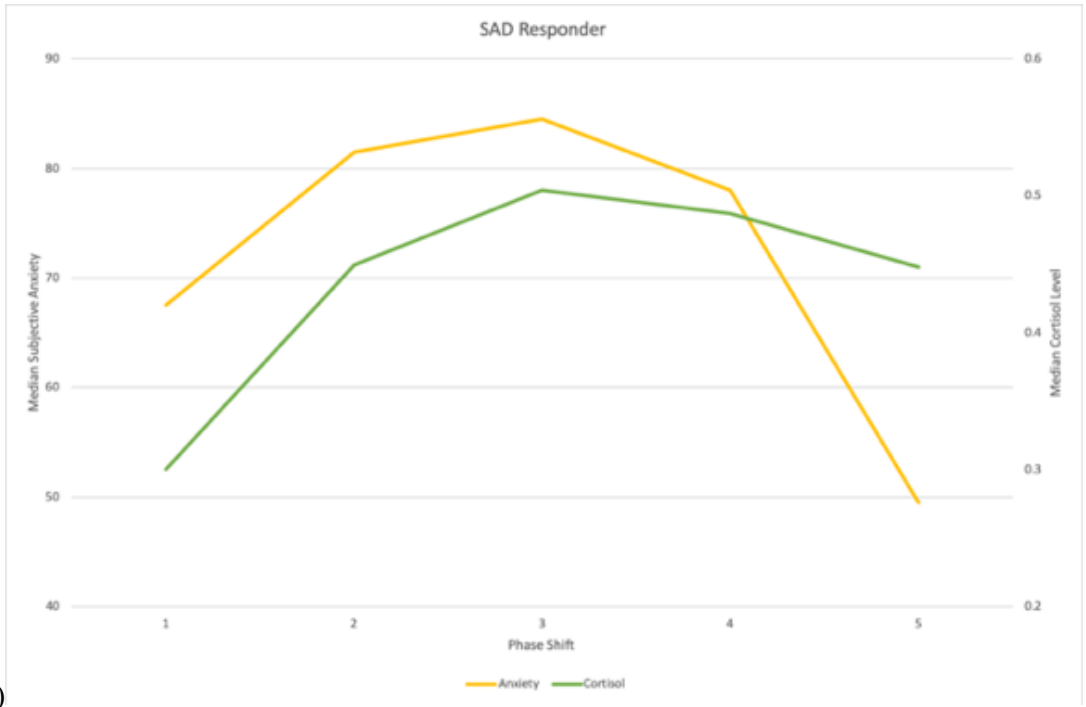


Figure 6.7. Median subjective anxiety report and salivary concentration (ug/dL) across time for (a) SAD responder and (b) HC responder.

a)



b)

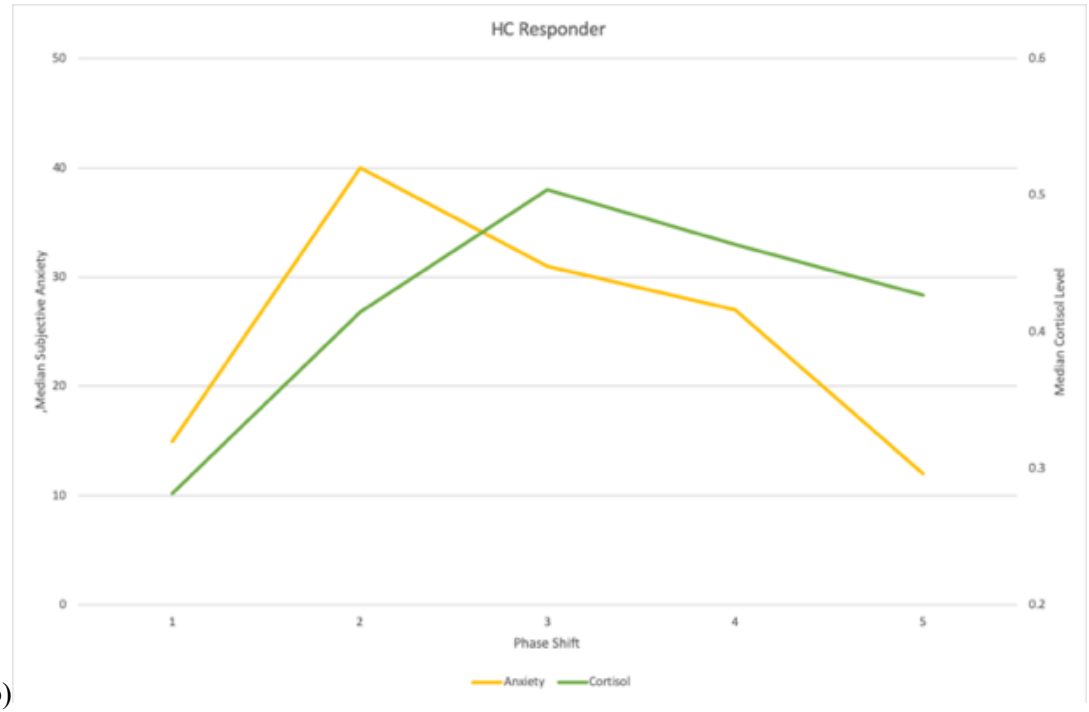


Figure 6.8. Median subjective anxiety and happiness report and salivary concentration (ug/dL) with phase shift for (a) SAD Responder and (b) HC Responder.

4. Discussion

The overarching goal of this study was to examine the subjective self-reported and physiological acute social stress response in people with SAD compared to

healthy controls. We aimed to contribute to the understanding of the subjective and physiological experience of acute social stress in SAD in the laboratory. As predicted, individuals with SAD did not differ from healthy controls in terms of salivary cortisol levels at baseline or in response to the acute lab stressor. Also in line with our hypothesis, individuals with SAD reported significantly higher levels of anxiety, sadness, tiredness, and withdrawal, and lower levels of happiness compared to healthy controls at baseline and overall across the stress protocol. Moreover, those with SAD also experienced greater overall intensity in anxiety and sadness over the stress protocol compared to healthy controls. When examining the within-person concordance between the subjective experience and physiological responding, we found evidence of a moderate positive association between cortisol and anxiety after correcting for the different temporal dynamics of subjective and physiological measures. Within-person concordance did not differ between the SAD and healthy control groups. Finally, as expected, cortisol responders did not differ from non-responders in salivary cortisol concentration at baseline, but showed significantly higher cortisol reactivity than non-responders during the stress task. In addition, cortisol responder and non-responder groups did not differ in their subjective experience at baseline or during the TSST.

4.1 Subjective but No Physiological Differences in Response to Social Stress Between SAD and Healthy Controls

Our finding of similar *baseline* salivary cortisol concentrations between the clinical groups is consistent with some previous studies (Uhde, Tancer, Gelernter, & Vittone, 1994; Van Veen et al., 2008), but diverges from Klumbies et al (2014) who found higher baseline salivary cortisol levels in those with SAD compared to healthy

controls. Several reasons may exist for the elevated baseline cortisol reported by Klumbies et al (2014) in contrast to our current findings. Firstly, this may have been due to the large number of SAD non-responders (39%) in the Klumbies et al (2014) study. Further, the Klumbies et al (2014) study included two time points in the baseline measure, i.e. at -45 min (upon arrival) and at -1 min (1 minute prior to task introduction). In the current study, our baseline measure was taken 5 minutes prior to task introduction (following a 15-minute acclimation period in the lab). From observation of the Klumbies et al (2014) study Figures, visually a significant decrease in salivary cortisol is observed in all groups (SAD-responders and non-responders and HC-responders and non-responders) from the start to the end of the waiting period, with the groups coming closer together at the end. This likely reflects a reduction in the impact of extraneous factors (e.g. running late, finding an unfamiliar location) on cortisol and thus the time point towards the end of the waiting period may be a more accurate measure.

Secondly, variations in the completion of informed consent and the proximity of this to the stress protocol may also be relevant to varying baseline measures. While often not explicitly reported, how participants are informed of the task and how close in proximity participants are made aware of the upcoming stress task may increase anticipatory anxiety, particularly among individuals with SAD. This may contribute to increases in cortisol responding, particularly in the clinical group, prior to commencement of the task. As the testing session for this protocol was part of a larger study, participants had previously completed informed consent and introductions approximately 5 days prior to their lab session and were already familiar with the sampling procedure as participants were required to provide saliva samples on other days prior to the TSST lab day.

Most interestingly, was the finding that there were also no group differences in the salivary cortisol response *across the stress protocol*. Our findings are consistent with studies reporting no difference in the salivary cortisol reactivity in response to a social stressor among individuals with SAD compared to controls (Klumbies et al., 2014; Krämer et al., 2012; Martel et al., 1999). However, our findings are also contrary to studies reporting significantly larger salivary cortisol reactivity to stress for those with SAD (see Furlan et al., 2001; Roelofs et al., 2009; van West et al., 2008).

Subjectively, and as expected, the SAD group reported significantly higher anxiety, sadness, withdrawal and tiredness, and lower self-reported happiness at baselines and evinced quicker changes (i.e., growth) in these subjective measures across the protocol. Anxiety and sadness were also experienced with significantly higher intensity in response to acute stress in the SAD group compared to the control group. These findings demonstrate that despite the SAD group subjectively differing compared to HC at baseline and in acute stress reactivity, this was not reflected in the physiological experience.

We cannot fully determine why our study results differ from those who did report cortisol differences between SAD and healthy control acute stress response. However, we took several measures to reduce the likelihood of potentially influencing factors. We employed an optimal version of the TSST in consideration of current research, using the shortest possible and most practical version of the TSST for logistical ease and reduced participant burden (see Chapter 5; Grace et al., 2019). We also strictly matched groups on characteristics such as age, gender, level of education and hormonal contraceptives, with no comorbidities existing in the clinical group (e.g. MDD or GAD), and all participants were medication free. We note medication use can contribute to variability in cortisol stress response. For example, in GAD,

treatment with common anxiolytics, such as escitalopram and diazepam, has demonstrated reduced cortisol secretion in response to acute stress (Plag et al., 2013). The presence of medication use in the aforementioned studies (e.g. medication use not reported, Beaton et al., 2006; Furlan et al., 2001; medication use included in sample, Roelofs et al., 2009; medication use screened for in healthy controls but not SAD, van West et al., 2008) may have contributed to the mixed results.

A further potential explanation for the mixed results in the literature is the high comorbidity of SAD with other disorders (Ruscio et al., 2008) and the impact of other mental health disorders on cortisol responding. SAD is highly comorbid with MDD and GAD particularly. Interestingly, MDD has been associated with blunted cortisol responding (Burke, Davis, Otte, & Mohr, 2005) and GAD has been associated with hyper-reactivity in cortisol response (Plag et al., 2013). As such, the presence of comorbidities in the above studies may also have influenced the findings. We report no comorbidities in our clinical sample, so these results may be a true reflection of the acute stress response in "pure" SAD. However, we note SAD is a highly comorbid disorder, and consideration of the impact of comorbidities on the experience of the disorder provides equally valuable information. Future research may look to examine the acute stress response in SAD, with and without comorbidities, or in direct comparison with other relevant primary disorders (e.g., GAD) in order to better understand differences in physiological responding to stress among anxiety/mood disorders.

Interestingly, despite the expected higher total growth and time-dependent reactivity observed in salivary cortisol secretion in responders compared to non-responders, baseline salivary cortisol did not differ according to cortisol responder status nor were any differences observed in the subjective reports of responder and non-responder groups. Few studies have examined determinants of cortisol response

and non-response to acute stress and why some individuals respond while others do not (Miller et al., 2013). Future research comparing the cortisol stress response between clinical groups should consider the influence of responder vs. non-responder subgroups. For instance, if non-responders are not completely omitted from analyses, then group mean salivary cortisol responses may be influenced by the increased secretion of cortisol in responders or the proportion of responders to non-responders in each group (Miller et al., 2013). Our samples size of ten SAD-NR and seven HC-NR would be insufficient to explore why some individuals respond while others do not. Future research with more adequate sample sizes may benefit from understanding determinants of salivary cortisol response to provide greater insight into the nature of the acute stress response. This could have important implications for understanding the relationship between physiological and subjective acute stress in a clinical population.

4.2 Concordance in Subjective and Physiological Acute Stress Responses in SAD

In addition to examining whether SAD and HC differed in their subjective and physiological acute stress responses, we also investigated whether concordance among the physiological and subjective stress responses differed between clinical groups. This was a novel approach, as previous studies (e.g., Klumbies et al., 2014) inferred discordance between physiological and subjective measures, without formally testing for this using temporal cross-correlations. In contrast, when we accounted for the different dynamics of physiological and subjective responses (Schlotz et al., 2008), we found evidence of response concordance (i.e., moderate-to-strong positive correlations between state anxiety and salivary cortisol) among participants in both the SAD and control groups. This is the first study to show concordance in subjective and physiological response systems to social stress in SAD and healthy controls. Our

analyses were based on a small number of time-points (T = 5) and assumed equal intervals between measurement occasions. Thus, future research would benefit from examining concordance between the subjective and physiological stress response across a broader range and number of time points (e.g. at baseline, across the entire stress protocol, and across recovery) with consistent time intervals between successive measurement occasions (e.g. 5 min intervals) or alternatively using a continuous time modelling approach (van Montfort, Kees, & Voelkle, 2018).

4.3 Theoretical and Diagnostic Implications

Cognitive models of SAD propose that when a socially anxious individual is faced with social threat, they shift their attention inward – engaging with increased self-monitoring and examination of their current experience (Hirsch, Clark, Mathews, & Williams, 2003). With our results indicative that SAD individuals experience the same physiological response to acute social stress as healthy controls, then it may be the cognitive interpretation of this stress response and the relationship SAD individuals have to their acute stress response that leads to the increased anxiety and negative affect and diminished positive affect observed. As suggested by Klumbies et al (2014) these results lend support to cognitive models of SAD in that it is not the physiological acute stress response that varies, but the negative interpretation of this stress response by those with SAD that lends to increased distress in the clinical group. The negatively biased processing of the social information and experience serve as a central mechanism in maintaining the disorder.

Further to this, existing evidence suggests that individuals with SAD exhibit greater anxiety sensitivity compared to controls (Anderson & Hope, 2009). Anderson and Hope (2009) found those with SAD were significantly more aware of

physiological arousal or changes in the system compared to healthy controls, and displayed higher ratings of anxiety in response to the experience of this physiological arousal. Interestingly, future research may look at whether the negative subjective experience varies according to R or NR status also and whether the physiological and subjective relationship varies between group and responder status in more adequate sample sizes. If these results support an interpretation bias in SAD individuals that contributes to increased distress in response to acute stress, then working towards normalising the acute stress response and the physiological experience of stress in the body (i.e. feelings of arousal, increased heart rate, sweating) in those with SAD, such that it is not experienced as unexpected or atypical to what stress will feel like in the body, may serve to reduce negative interpretations of the normal bodily experiences of stress.

Correct regulation of cortisol is essential to healthy functioning, as too little or too much in the system can potentially lead to serious health consequences (Adam et al., 2017). Both baseline cortisol and cortisol secretion in response to stress are carefully regulated in the HPA axis stress system, with this study demonstrating SAD did not reflect differences in both basal cortisol and cortisol reactivity in response to stress. Our results demonstrate that the physiological salivary cortisol response to acute stress in SAD is comparative to controls. When looking at the anxiety disorder umbrella, these results show that SAD participants do not display the extreme form of focused fear or threat reactivity you would observe in a specific phobia, nor did they display the defensive impairment seen in GAD or panic disorder (see Lang & McTeague, 2009). Lang and McTeague (2009) propose that SAD is a transition diagnosis on the anxiety spectrum, embedded between the extreme forms of focused fear reactivity observed in specific phobias, and the wide ranging frequent stress responses observed in the chronically anxious disorders of GAD and panic disorder.

Our findings support this view of SAD as a transition diagnosis, a disorder that does not physiologically demonstrate chronic overloading of the HPA axis system observed in GAD and panic disorder, nor does it represent extreme forms of focused fear reactivity.

Despite this, physiological and neurological differences in SAD have been found. In the brain, evidence has demonstrated central nervous system (CNS) hyperactivity in SAD, with Goldin et al (2009) demonstrating via functional magnetic resonance imaging that when compared to controls, those with SAD show increased negative emotion reactivity and reduced cognitive regulation-related neural activation when exposed to social threat stimuli. Physiologically, autonomic cardio regulation has also been shown as altered in SAD on the subjective, physiological and neurological level (see Gaebler et al., 2013; Pittig et al., 2013). The central nervous system (CNS) and the endocrine system are tightly interconnected to coordinate HPA axis activity in response to stress (e.g. glucocorticoid release), with the sympathetic branch of the autonomic nervous system (ANS) and its influence in cardio regulation also a common marker of stress (e.g. increased heart rate and blood flow in response to stress due to excitation of the cardiovascular system; Ulrich-Lai & Herman, 2009). With the HPA axis producing measurable changes in salivary cortisol in response to stress and the sympathetic-adrenal-medullary system producing epinephrine and norepinephrine and measurable changes in heart rate in response to stress, the two intersecting systems are associated with the normal stress response. In SAD, heightened CNS activity (e.g. amygdala activation in response to social threat; Evans et al., 2008; Goldin et al., 2009), and altered cardiovascular regulation associated with the sympathetic-adrenal-medullary system (Gaebler et al., 2013; Pittig et al., 2013) are observed, however, this hyperactivity is not observed in altered HPA axis activity in

response to social threat. Evidence from this study suggests a potential disconnect between the fast acting CNS and sympathetic-adrenal-medullary (SAM) system and the slow acting HPA axis threat response systems in SAD.

4.4 Conclusion

This study demonstrated that during social stress, those with SAD only differ from healthy controls on subjective stress reactivity, with no difference in acute salivary cortisol response. Moreover, this study demonstrated subjective stress (i.e. anxiety) was moderately concordant with salivary cortisol in response to stress in all participants, when accounting for the temporal dynamics in subjective and physiological stress response systems. These findings warrant further examination of the relationship between the subjective and physiological response to acute stress and how this informs acute stress reactivity. Along with existing evidence demonstrating CNS and ANS differences in SAD (i.e. amygdala hyperactivity and altered cardio regulation), the results of this study support theories of SAD that emphasise cognitive factors influencing the experience of the disorder, with SAD in this study primarily reflected in experiential dysfunction, rather than physiological differences observed in the salivary cortisol response to acute stress.

CHAPTER 7. Empirical Study 2 – Ecological Momentary Assessment in Social Anxiety Disorder: An Extended Look at the Daily Life Experience of Social Stress

Abstract

Background: Social stress and fear of negative evaluation are central to the experience of social anxiety disorder (SAD). Subjective and physiological responses to social stress in SAD research have been commonly studied, though results have been mixed. Existing studies have largely been confined to the laboratory setting, and have relied on analogue samples, retrospective questionnaires, and laboratory observations. Few studies have examined both subjective and physiological responding to social stress among individuals with social anxiety disorder across time and contexts. Using a combination of experimental and naturalistic, ambulatory assessment methods, this study examines the daily life patterns of subjective and physiological responses to social stress in those with SAD, during the anticipation of, and recovery from, an acute social stressor.

Methods: We examined 40 individuals with a primary diagnosis of social anxiety disorder (50% female) and 41 age- , sex-, and education-matched healthy controls during the anticipation of and recovery from an acute social stress task across eight days. Specifically, an experimental induction of social stress was embedded within an 8-day ecological momentary assessment (EMA) design. Participants first completed a baseline EMA assessment for two days, after which they were informed about an upcoming social stress task on Day 3, which initiated the anticipation phase. On Day 5 participants attended a lab session to complete the Trier Social Stress Test, a widely-used and reliable induction of acute social stress. Post-stressor two additional two days of EMA assessment were completed to mark the recovery phase. Subjective responding to the social stressor in daily life was assessed using repeated ratings of momentary affect, self-esteem, threat awareness and environment security over the 8-day EMA protocol. To assess physiological responding, participants completed ambulatory salivary cortisol sampling repeatedly over three days (one day for baseline, anticipation and recovery phases, respectively). This allowed us to examine both subjective and physiological responding to a standardised social stressor during both anticipatory and recovery phases, which we compared to baseline functioning.

Results: Across all phases, those with SAD demonstrated higher levels of negative affect, lower levels of positive affect, lower self-esteem, higher fear of negative evaluation and less security in their environment compared with healthy controls. In the lead up to and recovery from a social stress task the SAD group also demonstrated more anxiety and less happiness and satisfaction with their appearance compared to their baseline assessment and healthy controls. Individuals with SAD also reported elevated anger in the recovery phase compared to baseline assessment and healthy controls. No physiological cortisol differences between SAD and healthy adults were found across any of the diurnal cortisol measures, though an overall higher level of average daily cortisol was found in the baseline phase for both groups compared to average daily cortisol found in the anticipation and recovery phases.

Conclusions: In assessing the changes in the subjective experience of those with SAD surrounding an acute social stressor, this research demonstrates that while the physiological profile of SAD is similar to healthy adults, the higher negative and lower positive subjective experience known to SAD is pervasive across time, with the subjective impact of acute stress increasing in the lead up to a social stressor and remaining elevated during recovery from social stress. These findings provide new insights in the experience of SAD, particularly the broader profile of the subjective and physiological experience of acute social stress.

Keywords: diurnal cortisol, cortisol awakening response, HPA axis, humans, ambulatory assessment

1. Introduction

Social anxiety disorder (SAD) is characterised by clinically significant fear and anxiety toward the potential judgement, scrutiny, and negative evaluation of others during social engagement or interaction (American Psychiatric Association, 2013). The disorder has emerged as one of the most prevalent mental health disorders in Western culture (Stein & Stein, 2008), with an estimated 12-month prevalence of 4.2% of Australians and a lifetime prevalence of over 8% of Australians (Australian Bureau of Statistics, 2008). The disorder is associated with significant impairment in daily functioning and quality of life. SAD is often accompanied by behavioural dysfunction and is highly comorbid with other mental health disorders (Heimberg & Magee, 2014). The disorder has been widely explored in the laboratory setting across physiological, emotional, behavioural and cognitive measures (e.g. Dixon et al., 2019; Etkin & Wager, 2007; Heimberg & Magee, 2014; Jamieson et al., 2013; Schmidt, Richey, Buckner, & Timpano, 2009). While existing research has largely informed the understanding of SAD, much is still to be known about the experience of the disorder in daily life. In this study, we used daily life (a.k.a. ambulatory) assessment methods to explore the everyday subjective (e.g. affect and self-esteem) and physiological (salivary cortisol) responding of individuals with SAD to an acute social stressor.

The subjective experience of SAD has been largely characterised by the presence of high negative affect (e.g. anxiety, anger, sadness), decreased self-efficacy (e.g. confidence in abilities) and confidence (Kashdan & Roberts, 2004), with diminished positive affect (e.g. happiness) and expression of positive affect also associated with social anxiety (Chen et al., 2012; Eisner et al., 2009; Kashdan & Roberts, 2004; Kashdan, 2007; Kashdan & Steger, 2006). The disorder is also associated with negative interpretation biases, particularly in the social context (Huppert et al., 2003). Negative interpretation biases in SAD have been demonstrated in attention, memory and imagery, which contribute toward increased threat-

and anxiety-related cognitions and an increased interpretation of social interactions as threatening (Crisan et al., 2016; Heimberg & Magee, 2014). Accordingly, individuals with SAD frequently interpret social signals as indicative of rejection or negative evaluation due to negative self-beliefs and dysfunctional assumptions about others' expectations (Gilboa-Schechtman et al., 2017). These negative interpretation biases have been attributed to the maintenance of SAD (Vagos & Pereira, 2012) and are associated with the use of behaviours that contribute to the maintenance of social fears (Hirsch & Clark, 2004). The integral role of negative cognitions and evaluations about the self in the aetiology and maintenance of SAD has been emphasised in both cognitive models (Clarke & Wells, 1995) and new emerging models, such as the integrated aetiological and maintenance (IAM) model (Wong & Rapee, 2016) of the disorder. Wells and Clark (1997) proposed that negative automatic associations regarding the self (i.e. negative self-beliefs) are characteristic in SAD and influence the experience of the disorder. Individuals with SAD are shown to have lower levels of self-esteem, higher negative self-beliefs, and dysfunctional automatic associations with social cues (Dixon et al., 2019; Gilboa-Schechtman et al., 2017).

Physiologically, SAD has also been linked to dysfunction at multiple levels in response to social stressors (Crisan et al., 2016). Specifically, increased heart rate (Gerlach et al., 2001) and reduced heart rate variability (Gaebler et al., 2013; Pittig et al., 2013) in response to stress in SAD has been demonstrated, relative to healthy controls. Dysregulation in hypothalamic pituitary adrenal (HPA) axis response has also been linked to anxiety disorders (Elnazer & Baldwin, 2014), although specific evidence for HPA axis activity in SAD has been inconsistent (Crisan et al., 2016). There is no evidence of baseline HPA axis system dysregulation in SAD (Plag et al., 2013), however, in response to acute stress research has demonstrated similar (Klumbies et al., 2014; Levin et al., 1993; Martel et al., 1999), increased (Condren et al., 2002; Furlan et al., 2001; Roelofs et al., 2009) and decreased

(Beaton et al., 2006; Shirotsuki et al., 2009) cortisol concentration in individuals with SAD, compared with healthy controls. In Study 1 (see Chapter 6) we found that individuals with SAD showed similar acute physiological responses (in salivary cortisol) to healthy controls during a lab-based social stress induction. Few studies, however, have examined how individuals with SAD respond to a social stressor outside the lab, either in terms of physiology or subjective experience.

Our current understanding of SAD is largely based on laboratory research that relies on experimental methods and retrospective self-reports (Santangelo, Ebner-Priemer, & Trull, 2013). Yet, the symptomology of SAD is dynamic: the disorder is context dependent (i.e. fear or anxiety experienced is socially based) and time-contingent (i.e. unpredictable and variable across time), with the variant profile of the disorder significantly increasing the challenges of assessing the various psychological states and underlying mechanisms of SAD in the laboratory setting (Walz et al., 2014). Employing ambulatory assessment methods, such as ecological momentary assessment (EMA; i.e. naturalistic self-reported subjective states, behavioural assesment and physiological monitoring in daily life; Trull & Ebner-Priemer, 2014) allows for examination of psychological functioning across real-world contexts and time in daily life (Mehl & Conner, 2012). The repeated nature of EMA sampling could more accurately capture the context- and time-dependent experiences of those with SAD.

Despite EMA providing an innovative and novel way to explore psychopathology, few studies to date have explored SAD in daily life. Twelve existing studies have employed EMA in SAD (see Farmer & Kashdan, 2014, 2015; Faytout & Swendsen, 2009; Gloster et al., 2017; Goodman & Kashdan, 2019; Johnson et al., 2009; Kashdan & Farmer, 2014; Kashdan et al., 2014; Nylocks et al., 2018; Russell et al., 2011; Villanueva et al., 2019; Wilson et al., 2018). Collectively, these studies have contributed to a richer understanding of how SAD affects every day functioning. Of particular relevance to this study is EMA

research that has explored the level, reactivity and fluctuation of emotions and self-esteem across time in SAD (e.g. positive and negative affect), associations between social engagement and affect, and stress sensitivity and the response to stress in SAD.

To our knowledge, only one study to date has used EMA to explore self-esteem experiences in SAD across time (Farmer & Kashdan, 2014), despite models of SAD highlighting the role of reduced and changeable self-esteem in the phenomenology of the disorder (Clark & Wells, 1995; Moscovitch, 2009). Farmer and Kashdan (2014) found those with SAD reported lower self-esteem overall and displayed greater instability in self-esteem, though this was driven by mean level differences in self-esteem. The study also found individuals with SAD demonstrated higher levels of negative affect and increased instability in negative affect, with greater likelihood of acute moment-to-moment shifts in negative affective states, as well as difficulty in recovering from negative affect and maintaining positive affective states (i.e. problematic self-regulation)(Farmer & Kashdan, 2014). Exploration of positive affect revealed while positive affect was lower overall in SAD compared to healthy controls, positive affect was also relatively stable and did not demonstrate the same instability as negative affect across time.

Farmer and Kashdan (2015) also examined how strongly those with SAD react to social stress in daily life. They found in response to social stress in daily life, those with SAD responded more strongly in their negative affect responses compared to healthy controls, and that those with SAD reported interpersonal stress at an increased frequency when compared to healthy controls. Individuals with SAD also reported negative social experiences more frequently and demonstrated reduced reporting of meaningful positive social events in daily life. No evidence was found to support an increased likelihood for social stress to occur in SAD following intense negative affect in the days prior. The study concluded evidence for greater stress sensitivity in SAD in daily life, increased rigidity in reactions (i.e. consistently

high stress sensitivity) and greater reports of stressful experiences in the disorder compared to healthy controls (Farmer & Kashdan, 2015) and provides insights into the heightened stress sensitivity toward social engagement in SAD and increased generation in the disorder, along with reiterating the higher negative and fewer positive experiences common to SAD.

Through daily life assessment these EMA studies contribute novel understanding toward the daily life level and fluctuation of affect and self-esteem in SAD, how SAD individuals differ in their reactivity to social engagement, and how individuals with SAD exhibit greater stress sensitivity and stress generation in daily life. A limitation of most EMA studies, including those reviewed above, is that they do not control for individual differences in exposure to social experiences, which may vastly differ across participants. No study, to our knowledge, has looked at the temporal dynamics of affect and self-esteem among individuals with SAD surrounding a standardised social stressor.

However, Koval and Kuppens (2012) used a combination of lab and EMA methods to study individual differences in the daily affect dynamics of undergraduates during the anticipation of a standardised social stress induction. In this study, participants scoring higher on a fear of negative evaluation questionnaire showed larger changes in the temporal dynamics of negative affect leading up to a lab-based social stress task. These findings suggest that individuals with SAD may also show more pronounced changes in affect dynamics surrounding a social stressor. More broadly, this study demonstrates the feasibility of embedding a lab-based social stressor within a naturalistic EMA design. However, Koval and Kuppens (2012) focused exclusively on subjective affective experience during the anticipation of a social stressor and used an unselected undergraduate sample. Thus, it remains unknown how SAD influences other components of stress responding (e.g., physiological functioning) during both anticipation and recovery phases of a social stressor.

1.1 The Current Study

The present study examined the broader profile of the acute social stress response in SAD, collecting participant data using EMA techniques in daily life. The subjective experience of participants was captured via a purpose-built smartphone application installed on participants' own smartphone devices to assess subjective responding surrounding a social stressor in daily life, while ambulatory salivary cortisol sampling provided a physiological measure of changes in the diurnal cortisol rhythm surrounding the stressor. A standardised lab social stress induction was embedded within this EMA design: all participants completed the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) on day five of the study. This hybrid design capitalises on the strengths of both EMA and lab-based methods by capturing individual differences in daily stress responding to a standardised social stressor, while maximising ecological validity. The TSST, a widely-used lab stress induction protocol, is highly effective at inducing subjective and physiological stress responding as it involves both social evaluative threat (i.e., potential negative evaluation by others) and uncontrollability (Dickerson & Kemeny, 2004). We have developed an optimised protocol of the TSST (see Chapter 5; Grace et al., 2019), which was employed in the current study. The current study extends on the findings reported in the previous chapter (see Study 1, Chapter 6), which focussed on the acute stress response during the TSST protocol in the lab. Specifically, the current study focuses on subjective and physiological stress responding in daily life during the (i) anticipation of, and (ii) recovery from the TSST among individuals with SAD versus healthy controls. Subjective stress responding was operationalised by examining negative affect, positive affect, state self-esteem and threat awareness. Measures of affect included four negative affect (e.g. anxiety, sadness, embarrassment, anger) and one positive affect (e.g. happiness), with self-esteem (i.e. beliefs about self) measures relating to self-efficacy (e.g. confidence in abilities), appearance satisfaction and fear of negative evaluation also explored.

Threat awareness was measured according to attention toward other people's judgment or scrutiny (a core component of SAD) and environment security included the level of security in the environment at the time of report. Physiological stress responding was operationalised by examining changes across three components of daily cortisol concentration: diurnal cortisol slope, area under the curve and cortisol awakening response (CAR; a marked increase in cortisol secretion observed in the first hour post-awakening).

Overall, the current study aimed to reduce memory biases and increase not only the generalisability and ecological validity of the data but to also contribute to the understanding of how the experience of acute social stress may change across different times and contexts in SAD. There is little research exploring SAD outside of the laboratory and a lack of understanding toward the true experience of the disorder in daily life. No study to date has examined associations between an acute social stressor and the subjective and physiological response in the broader context in SAD (i.e. the days leading up to and recovery from an acute social stressor; see Figure 7.1). Assessment of the subjective and physiological acute social stress response in SAD across a broader time frame could potentially improve our understanding of how social stress impacts individuals with SAD in daily life, beyond the immediate experience of acute social stress captured in the lab. This may inform new therapeutic approaches and decisions regarding disorder management.

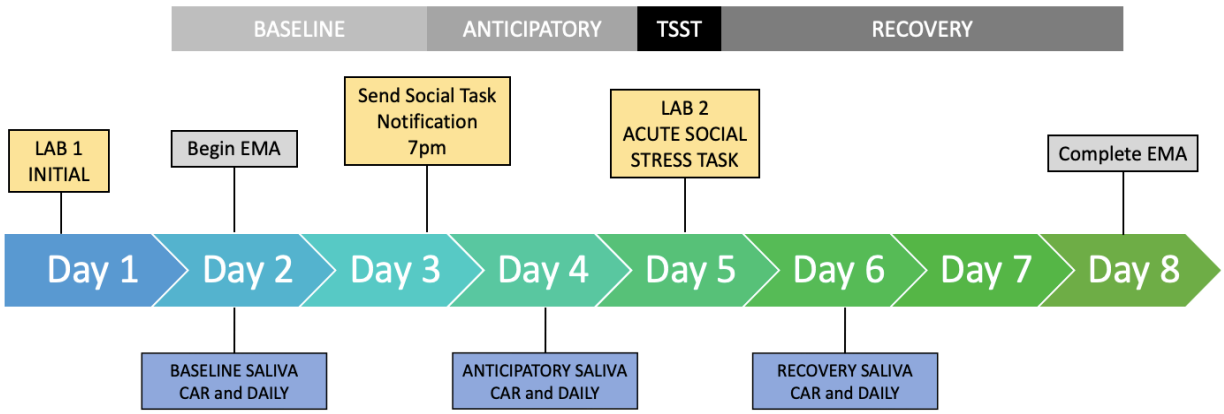


Figure 7.1. Overview of eight day testing protocol, baseline, anticipatory and recovery phases indicated by grey bars.

1.2 Aims and Hypotheses

The overarching aim of this study was to examine the effect of a standardized social stressor on subjective and physiological components of daily stress responding among individuals with SAD. Our specific aims were two-fold. First, we aimed to compare subjective experience of those with SAD and healthy controls, examining pre- and post-stressor changes in affect, self-esteem, threat awareness and environmental security, relative to baseline levels. We also examined the influence of other potentially modulating variables, such as sex, childhood mistreatment, attachment style and personality traits, on the subjective and physiological experience.

For aim one, we hypothesised a group difference across all phases of the protocol, such that participants in the SAD group would experience (a) higher levels of anxiety, sadness, embarrassment and anger and lower levels of happiness; (b) lower self-efficacy, dissatisfaction with appearance and higher concern with others opinion (hereafter *fear of negative evaluation)*; (c) greater attention toward others' judgement or scrutiny (hereafter *threat awareness*) and less security in their environment, compared with healthy controls. We also predicted a larger within-group phase effect for the SAD group, such that participants in the SAD group would show larger increases (relative to their baseline levels) than healthy controls in their self-reported (a) negative affect (i.e. anxiety, anger, embarrassment and sadness); (b) decreased positive affect (i.e. happiness); (c) reduced self-efficacy and security in the environment; and (d) higher fear of negative evaluation and threat awareness, during both the anticipation and recovery phases.

Second, the study aimed to examine associations between an acute psychosocial stressor and diurnal cortisol rhythm (CAR AUCi, daily AUCg and slope) in SAD in daily life, compared to healthy controls. For aim two, few studies address the topic of diurnal cortisol rhythm specific to SAD, as such we made no directional hypotheses and rather

conducted exploratory analyses investigating the effects of the TSST on cortisol functioning during the anticipation and recovery phases, relative to baseline, among those with SAD compared to healthy controls.

2. Methods

2.1 Participants

Forty SAD participants (50% females) and $n = 41$ healthy controls (48.80% female) comparable in age, sex and total years of education participated in this study (see Table 7.1). Participants were recruited via community-based advertising (online-advertisement; face-to-face; flyers). Participants for both the SAD and healthy control groups were required to be 18 – 55 years of age, be non-smoking, no substance abuse and free of psychotropic medication.

Social anxiety disorder is commonly associated with comorbid disorders, such as GAD and MDD or low mood. It was pre-determined that the presence of GAD and symptoms of low mood (though not a current depressive episode) would be accepted if secondary to a SAD diagnosis. No participants in the current study met a comorbid diagnosis. Participants with a co-morbid or primary diagnosis of a current acute depressive episode, alcohol/substance abuse, bipolar disorder, schizophrenia and post-traumatic stress disorder (PTSD) were excluded. The presence of any other clinically significant medical (e.g. diabetes, cancer), neurodevelopmental disorder (e.g. Attention deficit / hyperactivity disorder) or neurological condition excluded individuals from participation. Control participants were to have no current or suspected diagnosis of mental illness (screened via the MINI 7.0.2 screening tool and full MINI 7.0.2 if required) or self-reported neurological disorder.

To assess for potentially moderating factors in each group, all participants completed an assessment of perceived childhood mistreatment (e.g. Childhood Trauma Questionnaire;

Bernstein et al., 1994), attachment (e.g. Attachment Style Questionnaire; Van Oudenhoven, Hofstra, & Bakker, 2003) and personality (e.g. the Big Five Inventory; John et al., 2008). Table 2 presents the clinical characteristics of SAD and healthy control groups. At the time of participation, no participants were known to be engaged in psychotherapeutic intervention. All participants were required to speak English fluently (e.g. English as a first language or greater than 10 years speaking English and classifying themselves as fluent). Participants were required to be available between business hours and able to attend the compulsory 95 minute laboratory session on day five of the eight day protocol.

Table 7.1

Group Demographic Characteristics and Cortisol Responder Status of Social Anxiety Disorder (SAD) and Healthy Control (HC) Participants

N = 81		SAD (*n* = 40)	HC (*n* = 41)	Test	*p*	*df*
Gender (Female)	*n* (%)	20 (50.0)	20 (48.8)	$\chi^2 = .01$	.913	1
Age (Years)	*M* (*SD*)	28.42 (7.90)	25.75 (6.36)	*t* = 1.68	.098	79
Education (Total Years)	*M (SD)*	16.18 (2.25)	16.57 (2.09)	*t* = .83	.412	79
Education (Level Completed)				*FE*	.617	
Primary and Secondary School	*n (%)*	9 (22.5)	14 (34.1)			
TAFE, Certificate, Diploma	*n (%)*	5(12.5)	3 (7.3)			
Tertiary Degree	*n (%)*	21 (52.5)	18 (43.9)			
Postgraduate Tertiary Degree	*n (%)*	5 (12.5)	6 (14.6)			
Cortisol Non-Responders	*n (%)*	10 (25.0)	7 (17.1)	$\chi^2 = .77$	.381	1

Notes: M(SD) = mean (standard deviation), *n* (%) = number (percentage), df = degrees of freedom, SAD = social anxiety disorder, HC = healthy control, χ^2 = chi-square test, *t* = independent samples *t*-test, *FE* = Fisher's exact test of independence, *p* = *p*-value significant at $p < 0.05$ level, * $p < .05$. ** $p < .01$. ***$p < .001$

Table 7.2

Group Clinical Characteristics of Social Anxiety Disorder (SAD) and Healthy Control (HC) Participants

N = 81		SAD (*n* = 40)		HC (*n* = 41)		Test	*p*	*df*
Social Anxiety Severity								
SIAS	*M (SD)*	57.00	(9.26)	16.08	(10.30)	*t* = 18.69	< .001***	78
LSAS	*M (SD)*	78.70	(19.05)	24.26	(16.25)	*t* = 13.21	< .001***	73
Psychological Variables								
STAI (Trait)	*M (SD)*	55.10	(7.58)	36.05	(6.93)	*t* = 11.67	< .001***	77
DASS-21 Depression	*M (SD)*	17.00	(8.61)	3.56	(3.62)	*t* = 9.20	< .001***	79
DASS-21 Anxiety	*M (SD)*	18.85	(8.18)	4.15	(5.34)	*t* = 9.61	< .001***	79
DASS-21 Stress	*M (SD)*	24.70	(8.16)	8.29	(5.96)	*t* = 10.35	< .001***	79
RSE	*M (SD)*	14.15	(4.32)	22.49	(4.34)	*t* = 8.67	< .001***	79
Attachment - ASQ								
Confidence	*M (SD)*	25.58	(6.36)	35.46	(5.41)	*t* = 7.35	< .001***	75
Relationship as Secondary	*M (SD)*	20.32	(5.13)	16.03	(5.11)	*t* = 3.65	< .001***	74
Need for Approval	*M (SD)*	30.05	(4.22)	20.74	(6.15)	*t* = 7.72	< .001***	75
Discomfort with Closeness	*M (SD)*	43.00	(7.69)	32.13	(7.87)	*t* = 6.17	< .001***	76
Relationship Preoccupation	*M (SD)*	33.67	(6.66)	24.28	(6.77)	*t* = 6.17	< .001***	76
Personality - BFI								
Openness to Experiences	*M (SD)*	3.82	(0.58)	3.63	(0.62)	*t* = 1.37	.174	78
Conscientiousness	*M (SD)*	3.22	(0.60)	3.76	(0.66)	*t* = 3.83	< .001***	78
Extraversion	*M (SD)*	2.35	(0.66)	3.38	(0.72)	*t* = 6.68	< .001***	78
Agreeableness	*M (SD)*	3.48	(0.65)	3.88	(0.55)	*t* = 2.92	.005**	78
Neuroticism	*M (SD)*	3.94	(0.59)	2.61	(0.72)	*t* = 9.00	< .001***	78
Environmental - CTQ								
Emotional Abuse						FE	.002**	
None to Minimal	*n (%)*	17	(45.95)	33	(82.50)			
Low to Extreme	*n (%)*	20	(54.05)	7	(17.50)			
Physical Abuse						FE	.026*	
None to Minimal	*n (%)*	22	(57.90)	36	(87.80)			
Low to Extreme	*n (%)*	16	(42.10)	5	(12.20)			
Sexual Abuse						FE	.044*	
None to Minimal	*n (%)*	29	(74.35)	37	(92.50)			
Low to Extreme	*n (%)*	10	(25.65)	3	(7.50)			
Physical Neglect						FE	.181	
None to Minimal	*n (%)*	21	(53.85)	30	(73.17)			
Low to Extreme	*n (%)*	18	(46.15)	11	(26.83)			
Emotional Neglect						FE	< .001***	
None to Minimal	*n (%)*	11	(28.20)	30	(73.17)			
Low to Extreme	*n (%)*	28	(71.80)	11	(26.83)			
Minimisation Denial						FE	.043*	
None to Minimal	*n (%)*	33	(82.50)	26	(63.41)			
Low to Extreme	*n (%)*	7	(17.50)	15	(36.59)			

Notes: *M*(*SD*) = mean (standard deviation), *n* (%) = number (percentage), *df* = degrees of freedom, SAD = social anxiety disorder, HC = healthy control, SIAS = social interaction anxiety scale, LSAS = Leibowitz social anxiety scale, STAI = state trait anxiety inventory, DASS-21 = depression anxiety stress scale, RSE = Rosenberg self-esteem scale, ASQ = attachment style questionnaire, BFI = big five inventory, CTQ = childhood trauma questionnaire, *FE* = Fisher's exact test of independence, *t* = t-test of independence, *p* = *p*-value significant at $p < 0.05$. * $p < .05$. ** $p < .01$. ***$p < .001$

A total of 96 individuals were recruited for this study, with nine participants who did not commence their participation due to cancellation (n = 5), loss of contact (n = 2) and change of mind (n = 2), leaving 87 participants to commence participation. A further 6 participants did not complete their participation for various reasons (n = 2 refused the TSST, n = 1 due to illness, n = 1 left due to new work commitments, n = 1 displayed poor compliance and attendance, and n = 1 dropped-out). A final 81 participants comprising 40 SAD (20 females, mean age in years = 28.42, SD = 7.90) and 41 healthy controls (20 females, mean age in years = 25.75, SD = 6.36) remained for statistical analysis. The two groups did not differ significantly regarding their mean age, gender, or education level (see Table 7.1). Written informed consent was provided by all participants prior to their inclusion in the study and ethical approval for the conduct of the study was approved by the Australian Catholic University Human Research Ethics Committee.

2.2 Procedure

2.2.1 General overview. Participants completed an eight-day testing cycle involving three key parts (TSST; EMA and salivary cortisol). Two Lab-based sessions (Lab 1: 60 minutes in duration; Lab 2: 90-minutes in duration) were also completed (see Figure 7.1). Lab 1 involved completion of background and well- being questionnaires and participation set-up and study introduction, with the second lab involving completion of an acute social stressor (i.e. TSST; Kirschbaum et al., 1993). Participants completed seven-days of EMA using the smartphone application SEMA2 (Harrison et al., 2017) installed on participants' own Android or iOS device. SEMA2 prompted participants on their own personal smartphone device to rate their subjective experiences 10 times per day over seven days. The surveys were sent via fixed intervals of 72 MINS±20 MINS between the hours of 9am to 9pm. Participants also completed ambulatory saliva collection alongside the EMA protocol,

which involved three full days of saliva sampling on Days 2, 4 and 6 of the eight day protocol (see Figure 7.1). Participation was divided into three phases of participation: baseline, anticipation and recovery. Participants first completed baseline EMA assessment for two days following the initial lab visit. On the evening of day 3 of participation, participants were informed about an upcoming social stress task which initiated the anticipation phase of EMA assessment. On the afternoon of Day 5, participants completed the TSST to induce an acute social stress response. Following completion of the stressor, two full additional days of EMA assessment were completed to mark the recovery phase. This allowed us to examine anticipatory and recovery phases of stress responding and compare these to baseline functioning. See Figure 7.1 for overview of phases.

2.2.2 Scheduling of participants. The nature of the study design (phases and lab-based visits), meant that Lab 2 had to occur a minimum of five days post Lab 1 (i.e. acute social stressor completed on Day 5, with Lab 1 occurring on Day 1). The lab visits followed a particular sequence to accommodate sampling of the desired baseline, anticipatory and recovery phases of the testing cycle. Participants who were unable to complete the full 8-day cycle in one sequence were excluded. Due to fluctuations in female sex hormones during the menstrual cycle (see Chapter 5; Grace et al., 2019) female participants completed the TSST (Lab 2) while within the luteal phase of their menstrual cycle, i.e., between 14 to 28 days since the first day of the start of their last period of a regular 28-day cycle.

2.2.3 Communication across the protocol. Participants received a social task notification email in the evening of Day 3 (standardised to be sent at 7pm, with confirmation of receipt required to be sent by participants to the lead researcher via return email or text) in order to generate the anticipatory phase for the protocol. The notification email informed participants they would be completing a social task during Lab 2.

2.2.4 Subjective report – Ecological momentary assessment via smartphone. For the EMA component of the protocol, Participants were informed that each survey was time-stamped, with a 15-minute expiry in order to minimise retrospective recall bias. They were informed that they should complete each survey as close to the initial notification as was safely possible. Any survey not completed and submitted wholly before the 15-minute expiry would disappear and count as a missed survey. Items included in the ambulatory assessment component of the protocol were those measuring momentary affect, state self-esteem, threat awareness, and environment context (see Figure 7.2 for examples of questions).

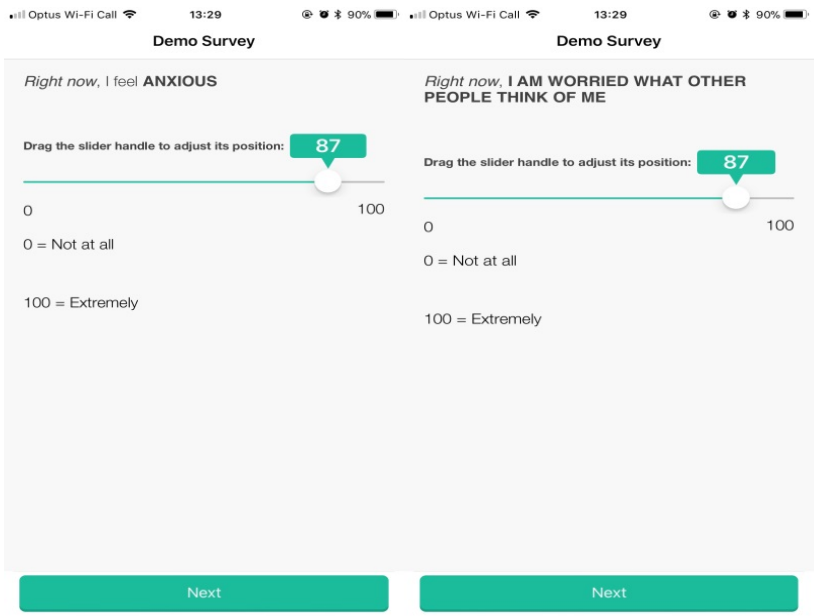


Figure 7.2. Example screenshots from the SEMA application

Momentary affect. Five questions captured momentary affect. These included four basic emotions, both negative and positive valance (one positive, e.g. *Right now*, how happy do you feel?; three negative, e.g. *Right now*, how [anxious/sad/angry] do you feel?) and one self-conscious emotion (negative valance, e.g. *Right now*, how embarrassed do you feel?). Responses were rated on a sliding scale from 0 (*not at all*) to 100 (*extremely*).

State self-esteem. Three questions captured state self-esteem. These included an appearance based self-esteem item (e.g. *Right now*, I am pleased with my appearance), a

performance based self-esteem item (e.g. *Right now*, I feel confident about my abilities) and a social based self-esteem item (e.g. *Right now*, I am worried what other people think of me). Response options were rated on a sliding scale of 0 (*not at all*) to 100 (*extremely*).

Attention toward threat. One question captured threat reactivity in regard to attention toward threat in the environment (e.g. *Since the last survey*, I have noticed people judging or scrutinising me). The response was rated on a sliding scale of 0 (*not at all*) to 100 (*extremely*).

Environment security. One question captured environment comfort (e.g. How secure have you felt in the environment you have spent most of your time?). The response was rated on a sliding scale of 0 (*not at all*) to 100 (*extremely*).

Participant reimbursement and compliance. To maximise EMA compliance, participant compensation for the full study protocol was provided up to $150, with a minimum amount of $130. Financial compensation was calculated on a pro-rata basis dependent upon survey compliance (e.g. response rate percentages of completed surveys; structured in a similar manner to previous studies, see Kashdan & Farmer, 2014). Maximum payment was provided if participants held a completed survey compliance between 70-80%, with compliance rates < 70% incurring the $20.00 deduction in final payment. Payment was provided in the form of gift vouchers. The mean number of surveys received was 75.48 (*SD* = 6.29; range 60-95) and participants completed an average of 79.35% (*SD* = 11.42%; range = 51-100%) of all scheduled surveys.

2.2.5 Physiological sampling. To capture the diurnal cortisol indices effectively, this study followed expert consensus guidelines for cortisol awakening response assessment (Stalder et al., 2016) and guidelines for diurnal cortisol indices sampling provided by Hoyt et al (2016). Participants were provided with pre-prepared ambulatory saliva sampling kits during their Lab 1 visit. The saliva kits contained a sample box containing 33 sample vials,

clearly labelled for each of the collection days – with each saliva vial also individually labelled, and a sampling record booklet. Upon completion of salivary cortisol sampling, participants were instructed to return their collection kit and record booklet via a pre-paid express post satchel provided during the Lab 1 visit. Participants sampled seven across the day salivary cortisol collections between the hours of 8am and 10pm (i.e. 0800hrs; 1000hrs; 1200hrs; 1500hrs; 1800hrs; 2000hrs; 2200hrs) and their CAR (4 samples collected at 0, 30, 45 and 60 minutes post awakening). The three sampling days were in accordance with the specific design phases of the protocol – baseline cortisol (Day 2; e.g. no awareness of the upcoming acute social stress task); anticipatory cortisol (Day 4; e.g. awareness of social task); and recovery cortisol (Day 6; e.g. post-completion of social task). For convenience, minimal participant burden and the benefit of no specialised training being required, samples were collected by absorbing saliva onto an oral swab (i.e., the SOS SalivaBio Oral Swabs from Salimetrics). As cortisol was the only analyte of interest for this study, the SOS swabs were a suitable choice for ease of use for multiple collections and for analysis of cortisol only. Participants were instructed to be as accurate as possible and to carefully track the specific details of their sampling and report any deviations from the expected sampling in their record book.

Returned participant saliva samples were stored at -80 degree Celsius until analyses. Samples underwent one freeze thaw cycle. For analyses, samples were thawed and analysed using commercially available kits (Salimetrics, USA) according to manufacturer instructions (Stratech Scientific APAC PTY Ltd). The Salimetrics cortisol assay kit is a competitive immunoassay specifically designed to measure salivary cortisol. Thawed samples were centrifuged at 1500 x *g* for 15min to collect clear saliva and this saliva was used without further processing for all assays. All samples were brought to room temperature before adding to the assay wells, with all samples analysed in duplicate. Intra- and inter-assay

coefficients of variation were at 4.3% and 4.6 % respectively for salivary cortisol. Salivary cortisol correlates well with matched serum cortisol concentrations; r = .91, assay sensitivity equal to .003μg/dL. All analyses were within set proficiency standards. Nine samples had insufficient volume for analysis across all participants. Inhouse methods of minimum sample recovery were tried however analysis was not able to be completed (noted as missing samples). Where appropriate, missing samples were replaced with statistical analyses (see statistical analyses). Final salivary cortisol analyses included 40 SAD and 40 healthy controls. Figure 7.3 provides a summary of all measures collected across the three phases.

2.2.6 Acute psychosocial stressor – The Trier Social Stress Test. During the second laboratory visit on Day 5, participants completed the TSST (Kirschbaum et al., 1993). For the detailed TSST methodology utilised for this study, see Chapter 5 (Grace et al., 2019). Study 1 (Chapter 6) reports on the subjective and physiological outcomes of the TSST not reported here. Because the current study intended to investigate the physiological response to an acute psychosocial stressor in the broader context, participants who failed to demonstrate a significant physiological stress response (i.e. cortisol non-responders; n = 17) during the TSST in Study 1 were excluded from further physiological analyses, resulting in n = 64.

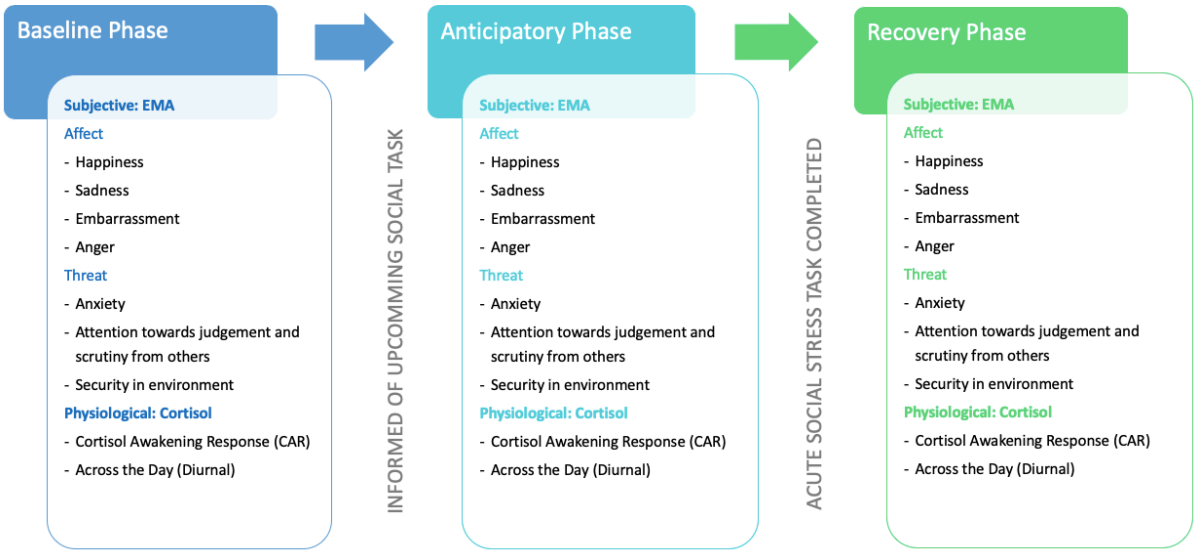


Figure 7.3. Summary of subjective and physiological measures collected across phases

2.3 Statistical Analysis

Statistical Package for the Social Sciences (SPSS; IBM Corp, 2015), Stata statistical software (StataCorp, 2019) and R (R Core Team, 2014) using the interface RStudio (RStudio Team, 2015) were used to perform all statistical analyses for this study.

2.3.1 EMA data analysis – Multilevel mixed-effects modelling of intensive longitudinal data.

Data pre-processing to check for missing values and outliers and was conducted in R (R Core Team, 2014) using the *cleansema* package (Murphy, 2017). Following McCabe and Fleeson's (2012) guidelines, EMA items with reaction times of less than 500*ms* were considered careless/random responding and were replaced with missing values (n = 120; .14% of all completed responses). After data cleaning, a total of 6,114 EMA surveys were included in the final analyses, made up of 4,823 completed surveys with answers and 1291 missing surveys.

Analysis of EMA data was completed primarily via multilevel (i.e. between and within) mixed-effects (i.e. fixed and random effects) modelling, which accounts for the hierarchical structure of the data, in which measurement occasions (i.e., EMA surveys, n = 6114) were nested within participants (n = 81). Our primary analyses focused on modelling the impact of an acute stressor (TSST) on subjective experience during the anticipatory and recovery phases (relative to baseline) among individuals with SAD vs. healthy controls. To model the effect of the TSST on the subjective variables of interest, we ran a separate multilevel mixed-effect model for each subjective outcome that included fixed-effects of "phase" coded as a three-level categorical variable (0 = baseline; 1 = anticipation; 2 = recovery) and group as a dummy-coded variable (0 = HC; 1 = SAD). To model group differences in terms of stress responding during the anticipation and recovery phases (vs. baseline) we also modelled the cross-level interaction between group and phase. Method of

estimation used to produce parameter estimates was maximum likelihood (ML), with ML better for unbalanced data. Stata uses ML by default and was utilised for these analyses.

Model 1 included fixed-effects of phase, group, and phase by group cross-level interaction, with weekend also included (to account for weekend differences). We specified random-effects at the participant level (i.e. Participant ID). Results are reported in Table 7.5 and 7.6. The subjective outcomes of interest included four negative affect (e.g. anxious, sad, embarrassed, anger) and one positive affect (e.g. happy)**,** three measures of state self-esteem (e.g. self-efficacy, fear of negative evaluation and appearance satisfaction), one measure of threat awareness (e.g. awareness of judgement or scrutiny by others) and environment comfort (e.g. security in environment). Despite weekend not being specifically of interest, due to prior knowledge of the potential influence of weekend, we control for it potentially influencing the patterns observed (Mehl & Conner, 2012). We also tested whether the addition of childhood maltreatment (i.e. CTQ factors), attachment style (i.e. ASQ factors) and personality (i.e. BFI factors) into separate models (for each measure, and each factor) influenced the primary subjective measures.

2.3.2 Diurnal cortisol analysis. Data was first checked for missing values and outliers. Where data was not normally distributed, non-parametric tests were used. Non-parametric procedures do not assume the sample population is normally distributed and provide useful analyses where violation of normality assumptions would make interpreting parametric tests problematic. For salivary cortisol CAR, if there were ≤ 1 (out of four) salivary cortisol CAR values missing for each phase, then they were replaced with the expectation maximisation algorithm. If there were > 1 (of the 4) CAR samples missing, the phase was excluded from further CAR analyses (baseline CAR $n = 2$; anticipatory CAR $n = 1$; recovery CAR $n = 2$). For diurnal cortisol (across the day sampling), if there were ≤ 2 (out of the seven) salivary cortisol sample values missing for a participant phase, then values were

replaced using linear transformation. If > 2 (out of the seven) across the day samples were missing, then the phase was excluded from further analyses (baseline diurnal $n = 3$; anticipatory diurnal $n = 1$; recovery diurnal $n = 3$). Outliers in salivary cortisol with z-scores of more than +/-3 standard deviations were excluded from any physiological statistical analyses (baseline CAR and diurnal exclusions $n = 3$; anticipatory CAR and diurnal exclusions $n = 1$; recovery CAR and diurnal exclusions $n = 3$). For analyses of slope, outliers with z-scores of more than +/- 3 standard deviations were excluded from any physiological statistical analyses, this resulted in $n = 1$ participant slope being excluded from further salivary cortisol analyses where appropriate. The significance level was $\alpha = 0.05$ for all analyses (two-tailed).

Hoyt et al (2016) describe capture of the key characteristics of the diurnal cortisol rhythm involves analyses of the diurnal cortisol slope, area under the curve (AUC) and cortisol awakening response (CAR). This study examined these three key characteristics of the diurnal cortisol between groups and phases. Repeated sampling over time was employed to capture changes in salivary cortisol during the CAR (four timepoints) and across the day (seven timepoints) for each phase of the study (baseline, anticipation and recovery). The AUC was calculated for each participant's CAR (AUCi formula used) and across the day (AUCg formula used) saliva and then these measures were compared between groups. The AUC is commonly used in cortisol sampling in order to simplify the amount of data for statistical analysis and increase power of the testing (Pruessner et al., 2003). For detailed explanation of AUC, see Study 1 (Chapter 6). The AUC allowed for this study to monitor group and phase effects on salivary cortisol levels across time. To calculate the diurnal cortisol slope, regression based slopes were fit by regressing sample time on salivary cortisol concentration across the averages of each phase of data, as per Hoyt et al (2016).

3. Results

3.1 Subjective Report

Table 7.3 lists the median (Q1 to Q3) of each subjective variable at each study phase (baseline; anticipation; recovery) for each group (SAD vs. HC). Mann-Whitney *U* tests (with $\alpha = .05$) were used to determine whether each subjective variable differed between SAD and healthy controls at baseline and during the anticipation and recovery phases. Group baseline, anticipation and recovery phase Mann-Whitney *U* test comparison statistics and effect sizes are reported in Table 7.4. Intraclass correlation coefficients (ICC) calculated from intercept-only models (i.e. *null models*, which include no predictors) are reported for the ten subjective measures in Table 7.3. The ICC represents the proportion of between-person variance to total variance. The ICC statistic can be interpreted as a percentage, i.e. ICC = .35 indicates that 35% of the total variability in the measure was between persons and 65% was within persons.

SAD and healthy control groups differed on all subjective measures, during the baseline, anticipation and recovery phases (see Table 7.4). For affect, the SAD group experienced significantly higher anxiety, sadness, embarrassment and anger and lower levels of happiness compared to healthy controls during the baseline, anticipation and recovery phases. For self-esteem, the SAD group experienced significantly higher fear of negative evaluation, and significantly lower self-efficacy and appearance satisfaction compared to healthy controls during the baseline, anticipation and recovery phases. The SAD group also demonstrated significantly higher threat awareness and less environment security at baseline, and during anticipation and recovery, when compared to healthy controls.

3.1.1 Multilevel mixed-effects modelling to examine group differences in subjective stress responding during anticipation and recovery phases. Results from multilevel mixed-effects models for each subjective affect outcome, including group and phase main effects, group*phase interaction and weekend effect are reported in Table 7.5.

Results from multilevel mixed-effects linear regression models for each subjective self-esteem, threat and environment outcome including group and phase main effects, group*phase interaction and weekend effect are reported in Table 7.6.

Group differences. Across the seven-days, the pattern of baseline differences observed between SAD and healthy controls in the subjective measures (see Table 7.3 and 7.4) remained similar across the protocol overall when included in the mixed-effect model. A significant proportion of variance in all subjective measures across the protocol could be explained as a function of group status. Compared to healthy controls, the SAD group experienced significantly higher levels of anxiety, sadness, embarrassment and anger and significantly lower levels of happiness across all phases. The SAD group also experienced significantly higher fear of negative evaluation, and significantly lower self-efficacy and appearance satisfaction and also demonstrated significantly higher threat awareness and less environment security across all phases, compared to healthy controls.

Phase effect. Across all participants (compared to baseline) no anticipation phase effect was observed for any subjective measure. A phase effect was observed for happiness, anxiety and anger, with significantly higher reports of happiness, and lower reports of anxiety and anger occurring in the recovery phase compared to baseline, across the entire group.

Interaction effect – Phase x SAD. The interaction effect represents group differences in phase-related change (i.e., anticipation and recovery vs. baseline) for those with SAD compared with healthy controls. An interaction effect was observed for the subjective measures of happiness, anxiety, anger and pleasure with appearance. Specifically, in the anticipation phase, participants in the SAD group experienced greater increases in anxiety, and greater decreases in happiness and satisfaction with appearance (relative to their baseline levels) than participants in the healthy control group. In the recovery phase, participants in the SAD group continued to report more elevated anxiety and anger, and decreased happiness

and satisfaction with appearance (relative to their baseline levels), compared with participants in the healthy control group (see Table 7.5 and 7.6).

Weekend effect. Compared to weekdays, higher levels of happiness, self-efficacy and environment security were observed on the weekend, and lower levels of anxiety, fear of negative evaluation and threat awareness were observed across the entire group.

3.1.2 Multilevel mixed-effects modelling to examine effects of moderating variables and sex on subjective experience. The addition of the CTQ and ASQ factors and the BFI indices openness to experience, agreeableness and extraversion as fixed effects into separate models had no notable effect on the primary subjective measures group associations. The addition of the BFI indices neuroticism and conscientiousness as fixed effects had a significant effect on the group associations for anxiety, self-efficacy, fear of negative evaluation and appearance satisfaction. Specifically, neuroticism had a significant effect on the subjective measures of anxiety, $\beta = 6.712$, $SE = 2.82$, CI = 1.20 to 12.24, $p < .05$, self-efficacy, $\beta = -7.12$, $SE = 2.13$, CI = -11.31 to -2.94, $p < .001$, fear of negative evaluation, $\beta = 7.10$, $SE = 2.65$, CI = 1.88 to 12.30, $p < .001$, and appearance satisfaction, $\beta = -5.62$, $SE = 2.45$, CI = -10.41 to -0.83, $p < .05$. Conscientiousness had a significant effect on self-efficacy, $\beta = 11.022$, $SE = 2.05$, CI = 6.99 to 15.04, $p < .001$. We also examined sex differences within the SAD group and within the healthy control group. When compared to SAD females, males in the SAD group reported higher anger, $\beta = 12.95$, $SE = 4.72$, CI = 3.69 to 22.21, $p < .01$, embarrassment $\beta = 12.92$, $SE = 4.41$, CI = 4.28 to 21.56, $p < .01$, and threat awareness, $\beta = 11.12$, $SE = 4.48$, CI = 2.33 to 19.91, $p < .05$. These sex differences in the SAD male group did not appear when examined overall (SAD and healthy controls). Only one sex difference was observed in the healthy control group, with males reporting greater overall environment security, $\beta = 8.29$, $SE = 3.23$, CI = 1.97 to 14.62, $p < .01$, when compared to healthy control females.

Table 7.3

Median (Q1 to Q3) Subjective Report Measures for SAD and HC Across Phases of Participation and Intraclass Correlation Coefficients (ICC) for Null Models

Subjective Measure		SAD			HC			*ICC*
		Baseline	Anticipation	Recovery	Baseline	Anticipation	Recovery	
Anxious	Median (Q1 to Q3)	48 (18 to 69)	57 (22 to 70)	40.5 (18 to 69)	14 (0 to 29)	12 (0 to 27)	9.5 (0 to 26)	.43
Sad	Median (Q1 to Q3)	23 (6.5 to 45)	22 (7 to 40)	23 (9 to 50)	3 (0 to 16)	2 (0 to 17)	2 (0 to 15)	.38
Angry	Median (Q1 to Q3)	14 (0 to 30)	13 (0 to 29)	13 (0 to 35)	1 (0 to 16)	1 (0 to 13)	0 (0 to 12)	.42
Embarrassed	Median (Q1 to Q3)	17 (0 to 36)	19 (4 to 34.5)	18 (5 to 36)	1 (0 to 14)	1(0 to 14)	1 (0 to 14)	.40
Happy	Median (Q1 to Q3)	49 (30 to 64)	43 (26.5 to 63)	42 (25 to 63)	72 (61 to 83)	71 (59 to 81)	73 (62 to 83)	.34
Self-efficacy	Median (Q1 to Q3)	50 (29 to 65)	43 (28 to 62)	42 (26 to 61)	74 (64 to 82)	72 (62 to 81)	73 (63 to 83)	.42
Fear of negative evaluation	Median (Q1 to Q3)	36 (15 to 65)	40 (18.5 to 68)	31 (13 to 64.5)	9 (0 to 22)	7 (0 to 23)	6 (0 to 21)	.41
Appearance satisfaction	Median (Q1 to Q3)	47 (28 to 62)	41 (24 to 63)	39 (23 to 60)	72 (59 to 80)	69 (59 to 78)	71 (59 to 81)	.48
Threat awareness	Median (Q1 to Q3)	21 (0 to 50)	21 (4 to 45)	20 (4 to 45)	5 (0 to 19)	3 (0 to 18)	5.5 (0 to 20)	.31
Environment security	Median (Q1 to Q3)	70 (47 to 85)	68 (46 to 80)	65 (41 to 80)	85 (72 to 100)	85 (70 to 100)	82 (69 to 98)	.33

Note. Median (Q1 to Q3) = Median (interquartile range), SAD = social anxiety disorder, HC = healthy control, ICC = intraclass correlation coefficient

Table 7.4

Statistical Comparison of Group (SAD vs. HC) Subjective Report Measures During Baseline, Anticipation and Recovery Phases

Subjective Measure	Group Baseline Comparison (*n* = 1350 to 1362 surveys)				Group Anticipation Comparison (*n* = 1163 to 1173 surveys)				Group Recovery Comparison (*n* = 2132 to 2155 surveys)			
	z	*p*	*df*	*r*	*z*	*p*	*df*	*r*	*z*	*p*	*df*	*r*
Anxious	14.34	< .001***	1	.38	15.97	< .001***	1	.47	21.21	< .001***	1	.46
Sad	14.05	< .001***	1	.38	13.20	< .001***	1	.39	20.37	< .001***	1	.44
Angry	9.27	< .001***	1	.25	9.82	< .001***	1	.29	15.61	< .001***	1	.34
Embarrassed	12.50	< .001***	1	.34	12.91	< .001***	1	.38	18.29	< .001***	1	.39
Happy	17.70	< .001***	1	.48	17.49	< .001***	1	.51	25.50	< .001***	1	.55
Self-efficacy	19.55	< .001***	1	.53	20.04	< .001***	1	.59	27.00	< .001***	1	.58
Fear of negative evaluation	16.45	< .001***	1	.45	16.68	< .001***	1	.49	19.84	< .001***	1	.43
Appearance satisfaction	18.94	< .001***	1	.51	17.86	< .001***	1	.52	25.19	< .001***	1	.55
Threat awareness	10.68	< .001***	1	.29	11.45	< .001***	1	.34	14.22	< .001***	1	.31
Environment security	12.59	< .001***	1	.34	13.33	< .001***	1	.39	17.42	< .001***	1	.38

Note. SAD = social anxiety disorder, HC = healthy control, group phase comparison statistics based on a Mann-Whitney U test, *z* = z statistic, *r* = effect size, *n* = number of surveys included in phase analyses, *df* = degrees of freedom, *p* = *p*-value significant at $p < 0.05$ level, * $p < .05$. ** $p < .01$. ***$p < .001$

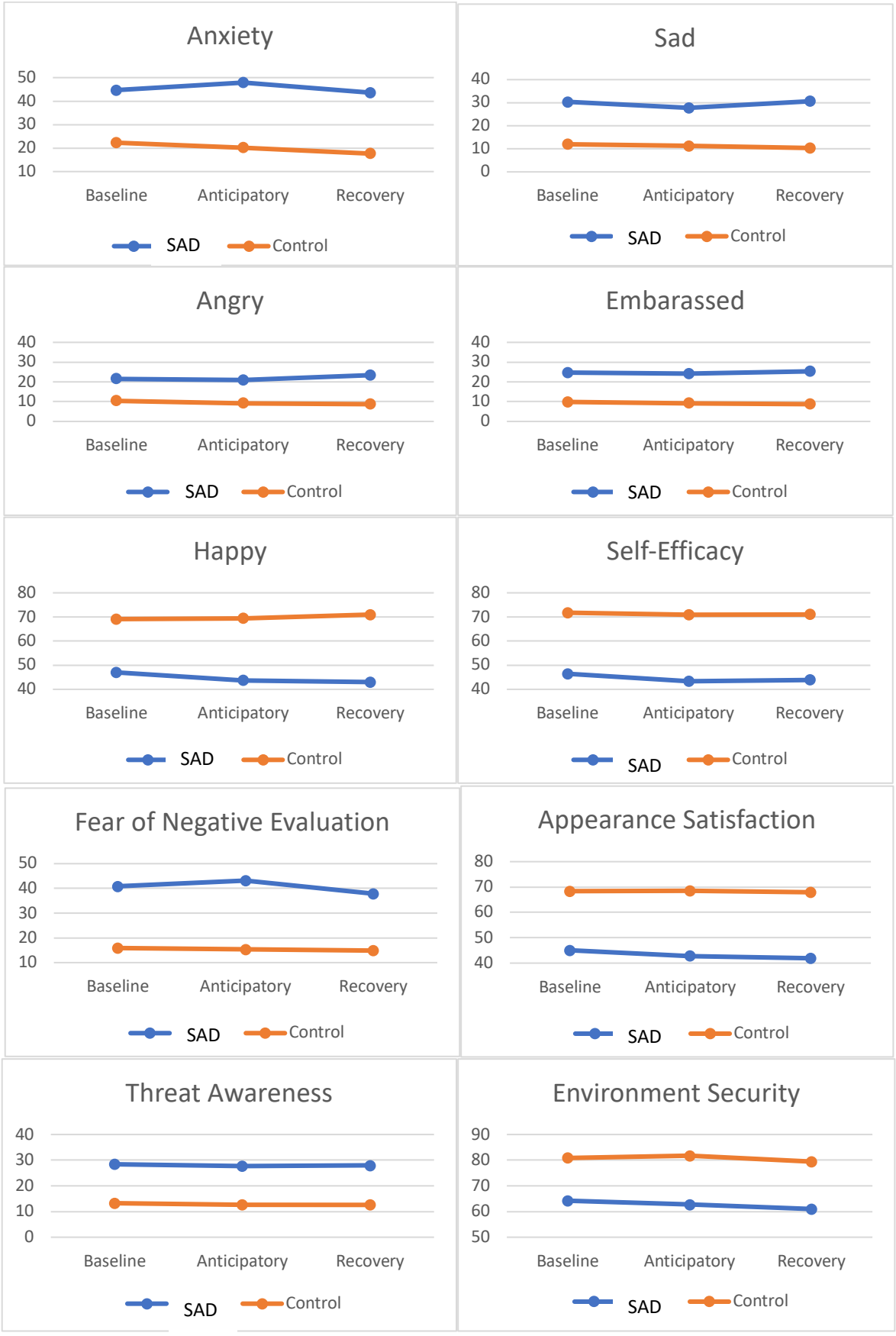


Figure 7.4. Model implied groups means (SAD vs. HC) for each subjective outcome at each study phase (baseline, anticipation, recovery).

Table 7.5

Results from Multilevel Mixed Effects Linear Regression for Each Subjective Affect Measure Accounting for Weekend

Subjective Affect	Parameter	Estimate (*SE*)	95% CI		*p*
			Lower Limit	*Upper Limit*	
Happy	Intercept	68.70 (2.10)	64.59	72.81	< .001***
N = 4684	Group main effect (SAD)	-22.09 (2.96)	-27.90	-16.28	< .001***
	Phase main effect (Anticipation)	.33 (1.01)	-1.65	2.31	.743
	Phase main effect (Recovery)	1.87 (0.88)	0.14	3.60	< .05*
	Interaction (SAD x Anticipation)	-3.61 (1.41)	-6.36	-0.85	< .01**
	Interaction (SAD x Recovery)	-5.88 (1.24)	-8.32	-3.44	< .001***
	Weekend	1.48 (0.58)	0.34	2.63	< .05*
Anxious	Intercept	23.25 (2.82)	17.72	28.78	< .001***
N = 4687	Group main effect – SAD	22.40 (4.00)	14.57	30.22	< .001***
	Phase main effect – Anticipation	-2.07 (1.15)	-4.32	0.18	.071
	Phase main effect – Recovery	-4.65 (1.01)	-6.62	-2.68	< .001***
	Interaction – SAD x Anticipation	5.26 (1.60)	2.13	8.41	< .001***
	Interaction – SAD x Recovery	3.57 (1.42)	0.80	6.35	< .05*
	Weekend	-3.49 (0.67)	-4.80	-2.19	< .001***
Sad	Intercept	11.93 (2.23)	7.57	16.30	< .001***
N = 4686	Group main effect – SAD	18.35 (3.15)	12.17	24.52	< .001***
	Phase main effect – Anticipation	-.70 (1.00)	-2.66	1.25	.479
	Phase main effect – Recovery	-1.61 (0.87)	-3.32	0.09	.064
	Interaction – SAD x Anticipation	-1.84 (1.39)	-4.56	0.89	.186
	Interaction – SAD x Recovery	1.91 (1.23)	-0.50	4.32	.120
	Weekend	0.13 (0.58)	-1.00	1.26	.823
Embarrassed	Intercept	10.07 (2.06)	6.03	14.10	< .001***
N = 4686	Group main effect – SAD	14.80 (2.91)	9.10	20.50	< .001***
	Phase main effect – Anticipation	-0.61 (0.89)	-2.35	1.13	.491
	Phase main effect – Recovery	-1.13 (0.78)	-2.65	0.39	.145
	Interaction – SAD x Anticipation	0.13 (1.24)	-2.30	2.56	.915
	Interaction – SAD x Recovery	1.81 (1.09)	-0.34	4.00	.100
	Weekend	-0.75 (0.51)	-1.76	0.25	.143
Angry	Intercept	10.34 (2.16)	6.11	14.57	< .001***
N = 4678	Group main effect – SAD	11.17 (3.05)	5.20	17.16	< .001***
	Phase main effect – Anticipation	-1.26 (3.05)	-3.00	0.49	.158
	Phase main effect – Recovery	-1.68 (0.78)	-3.21	-0.15	< .05*
	Interaction – SAD x Anticipation	0.63 (1.24)	-1.81	3.10	.613
	Interaction – SAD x Recovery	3.50 (1.10)	1.35	5.66	< .001***
	Weekend	0.23 (0.56)	-0.78	1.24	.656

Note. Estimates come from 5 models. SAD = social anxiety disorder, SE = Standard error, CI = confidence interval, Intercept = mean level of outcome at baseline for healthy control group, *p* = *p*-value significant at $p < 0.05$ level, * $p < .05$. ** $p < .01$. ***$p < .001$

Table 7.6

Results from Multilevel Mixed Effects Linear Regression for Each Subjective Self-Esteem, Threat and Environment Measure Accounting for Weekend

Subjective Measure	Parameter	Estimate (*SE*)	95% CI		*p*
			Lower Limit	*Upper Limit*	
Self-efficacy	Intercept	71.40 (2.14)	67.20	75.60	< .001***
N = 4682	Group main effect – SAD	-25.32 (3.04)	-31.26	-19.38	< .001***
	Phase main effect – Anticipation	-.85 (0.88)	-2.58	0.89	.338
	Phase main effect – Recovery	-.67 (0.78)	-2.19	0.85	.387
	Interaction – SAD x Anticipation	-2.25 (1.24)	-4.67	-0.17	.069
	Interaction – SAD x Recovery	-1.85 (1.09)	-4.00	0.29	.091
	Weekend	1.31 (0.51)	0.30	2.32	< .05*
Fear of	Intercept	16.53 (2.65)	11.34	21.72	< .001***
negative	Group main effect – SAD	24.90 (3.74)	17.55	32.23	< .001***
evaluation	Phase main effect – Anticipation	-.58 (1.12)	-2.77	1.61	.605
N = 4686	Phase main effect – Recovery	-1.04 (0.98)	-2.96	0.88	.290
	Interaction – SAD x Anticipation	2.95 (1.56)	-0.11	6.02	.059
	Interaction – SAD x Recovery	-1.89 (1.38)	-4.60	0.82	.172
	Weekend	-2.30 (0.65)	-3.58	-1.03	< .001***
Appearance	Intercept	68.12 (2.36)	63.48	72.75	< .001***
satisfaction	Group main effect – SAD	-23.25 (3.35)	-29.81	-16.69	< .001***
N = 4687	Phase main effect – Anticipation	.20 (0.88)	-1.51	1.92	.815
	Phase main effect – Recovery	-.37 (0.77)	-1.87	1.13	.627
	Interaction – SAD x Anticipation	-2.44 (1.22)	-4.84	-0.05	< .05*
	Interaction – SAD x Recovery	-2.77 (1.08)	-4.89	-0.65	< .01**
	Weekend	.69 (0.51)	-0.31	1.68	.175
Threat -	Intercept	14.27 (2.10)	10.16	18.38	< .001***
awareness	Group main effect – SAD	15.16 (2.96)	9.36	20.96	< .001***
N = 4649	Phase main effect – Anticipation	-.62 (1.08)	-2.72	1.50	.568
	Phase main effect – Recovery	-.60 (0.95)	-2.46	1.26	.525
	Interaction – SAD x Anticipation	-.08 (1.51)	-3.05	2.88	.957
	Interaction – SAD x Recovery	0.20 (1.34)	-2.43	2.82	.883
	Weekend	-3.70 (.63)	-4.93	2.47	< .001***
Environment	Intercept	80.27 (2.14)	76.08	84.46	< .001***
security	Group main effect – SAD	-16.71 (3.02)	-22.63	-10.80	< .001***
N = 4645	Phase main effect – Anticipation	0.83 (1.06)	-1.26	2.91	.437
	Phase main effect – Recovery	-1.48 (0.93)	-3.30	0.34	.112
	Interaction – SAD x Anticipation	-2.29 (1.48)	-5.20	0.61	.112
	Interaction – SAD x Recovery	-1.75 (1.31)	-4.33	0.82	.182
	Weekend	2.40 (0.62)	1.19	3.60	< .001***

Note. Estimates from 5 models. SAD=social anxiety disorder, *SE*=standard error, CI=confidence interval, *p* = *p*-value significant at $p < 0.05$ level, * $p < .05$. ** $p < .01$. ***$p < .001$

3.1.3 Subjective results summary. At baseline and overall across the protocol, compared to healthy controls, the SAD group experienced significantly higher anxiety, sadness, embarrassment, anger, fear of negative evaluation and threat awareness and significantly lower levels of happiness, self-efficacy, appearance satisfaction and environment security. In the anticipation phase (vs. baseline), those with SAD experienced higher anxiety, and lower levels of happiness and appearance satisfaction compared to healthy controls. In the recovery phase (vs. baseline) in SAD compared to healthy controls, with higher anxiety and anger, and lower levels of happiness and appearance satisfaction observed. Across the group higher levels of happiness, self-efficacy and environment security and lower levels of anxiety, fear of negative evaluation and threat awareness were observed on the weekend.

Neuroticism and conscientiousness moderated the subjective experience, with higher neuroticism associated with higher anxiety and worry for what others think and lower self-efficacy and appearance satisfaction. Higher conscientiousness was associated with higher levels of self-efficacy. Sex differences were apparent within groups, with higher anger, embarrassment and threat awareness observed in SAD males compared to SAD females. Males in the healthy control group reported greater overall environment security compared to healthy control females.

3.2 Physiological (Cortisol) Results

3.2.1 CAR AUCi. A 2x3 mixed model ANOVA was used to investigate the impact of an acute psychosocial stressor on the CAR AUCi of SAD individuals and healthy controls. For analyses, 59 participants had sufficient cortisol data to calculate the CAR AUCi statistic across three phases (i.e. baseline, anticipatory and recovery). The CAR AUCi was examined across three phases and the impact of group (i.e. SAD

vs. healthy controls) was investigated. The Shapiro-wilk, F_{max} and Levene's test statistics tested assumptions of normality and homogeneity of variance. The assumptions for a mixed model ANOVA were not violated. There was no significant main effect for phase, $F(2,114) = .142$, $p = .867$, partial $\eta^2 = .002$ with AUC_i not significantly differing in the baseline ($M = 5.40$, $SD = 11.92$), anticipatory ($M = 5.14$, $SD = 11.72$) or recovery phases ($M = 4.69$, $SD = 10.82$). There was also no significant main effect for group, $F(1,57) = 1.037$, $p = .313$, partial $\eta^2 = .018$. The phase by group was not significant, $F(2,114) = .860$, $p = .426$, partial $\eta^2 = .015$. Results remained similar when the covariates of perceived childhood maltreatment (e.g. CTQ factors), personality (e.g. BFI factors) and attachment (e.g. ASQ) were included in the model.

3.2.2 Across the Day AUCg. A 2x3 mixed model ANOVA was used to investigate the impact of an acute psychosocial stressor on the diurnal cortisol AUCg of SAD individuals and healthy controls. For analyses, 58 participants had sufficient data to calculate the diurnal cortisol AUCg statistic across three phases (baseline; anticipatory and recovery). Diurnal cortisol AUCg across the three phases and the impact of group (SAD vs. HC) was investigated. The Shapiro-wilk, F_{max} and Levene's test statistics were used to test the assumptions of normality and homogeneity of variance. The assumptions for a mixed model ANOVA were not violated. A significant main effect for phase was obtained, $F(2,112) = 3.225$, $p = .043$, partial $\eta^2 = .054$. Examination of the means indicated that baseline AUC_g was significantly higher ($M = 164.63$, $SD = 58.56$) compared to the anticipatory ($M = 148.31$, $SD = 48.71$) and recovery phases ($M = 152.18$, $SD = 47.90$). The was no significant main effect for group, $F(1,56) = .016$, $p = .899$, partial $\eta^2 = <. 001$. The was no significant interaction between phase and group, $F(2,112) = 1.345$, $p = .265$, partial $\eta^2 = .023$.

3.2.3 Across the Day Slope. The across the day slope was calculated for each participant across each phase. Between-group comparison using Mann-Whitney *U* tests indicated that the slope of across the day cortisol did not differ between groups (SAD vs. HC) at each phase; see Table 7.7. Within-group comparison using Kruskal-Wallis *H* tests showed no statistically significant difference in salivary cortisol slope across phases (baseline slope; anticipatory slope; recovery slope) within SAD or healthy controls, SAD, $\chi^2(2) = .187$, $p = .912$, and HC, $\chi^2(2) = .608$, $p = .738$.

Table 7.7

Median (Q1 to Q3) Across the Day Salivary Cortisol Slope for Group (SAD and HC) Across Phases

		SAD	HC	Test	*z*	*p*	*r*
Baseline Slope *N* = 63 SAD=30	Median (Q1 to Q3)	-.05 (-.08 to -.02)	-.05 (-.07 to -.03)	*U* = 457.00	-.52	.601	.07
Anticipatory Slope *N* = 64 SAD = 30	Median (Q1 to Q3)	-.05 (-.07 to -.03)	-0.06 (-.08 to -.04)	*U* = 413.00	-1.31	.192	.16
Recovery Slope *N* = 62 SAD = 28	Median (Q1 to Q3)	-.05 (-.07 to -.04)	-.06 (-.07 to -.04)	*U* = 423.00	-.97	.334	.12

Note. Median (Q1 to Q3) = median (interquartile range), SAD = social anxiety disorder, HC = healthy control, *U* = Mann-Whitney *U* test, *r* = effect size, *p* = *p*-value significant at $p < 0.05$ level

3.2.4 Physiological Results Summary. Physiologically, no group differences were found across measures of salivary cortisol CAR, AUC_g and slope. A phase effect was observed for baseline salivary cortisol across the day, with baseline AUC_g significantly higher than salivary cortisol total growth in the anticipation and recovery phases, though this did not differ between groups (SAD vs. HC).

4. Discussion

The overarching goal of this study was to examine the daily life experience of social stress in SAD. We aimed to contribute to the understanding of how the subjective and physiological responding to an acute social stressor in SAD unfolds in daily life. Compared to healthy controls, participants with SAD differed in their subjective experience at baseline, and in the days leading up to and following a social stress task. Those with SAD showed higher anxiety, sadness, embarrassment, anger and fear of negative evaluation, along with less happiness, self-efficacy and appearance satisfaction at baseline. These deviations from the typically healthy adult reports of affect, self-esteem, threat awareness and environment security were also observed across all phases of the daily life sampling. Individuals with SAD also experienced higher levels of anxiety, less happiness and reduced appearance satisfaction in the lead up to the social stress task. Following completion of the social stress task those with SAD again continued to report higher anxiety, lower levels of happiness and less appearance satisfaction, whereas healthy controls did not. Additionally, the SAD participants displayed more anger during the recovery from the social task, when compared to baseline measures and healthy controls.

A number of gender differences were also found within the SAD and healthy control groups, that were not present in the overall population. The SAD males had significantly higher levels of overall anger, embarrassment and threat awareness when they were compared to the females in their group specifically. While males in the healthy adult group demonstrated higher levels of overall environment security compared to females in their group, an effect was not present in the SAD group. These findings demonstrate the importance of exploring sex differences in the experience of mental health, with varying profiles in the experience of acute social stress differing according to sex. Furthermore, when accounting for other potential

factors that potentially influenced the subjective experience, individual's with higher neuroticism demonstrated higher levels of overall anxiety and fear of negative evaluation, with less self-efficacy and appearance satisfaction. Participants with higher conscientiousness reported higher levels of self-efficacy. The higher negative affect, along with lower positive affect and self-esteem and a heightened fear of negative evaluation are well known to SAD. However, these results demonstrate in part the shifting nature of affect, self-esteem, threat awareness and environmental security around completion of a social stress task in SAD. This study may add to the understanding of how the subjective experience and changes in the subjective experience contribute to maladaptive psychological states and behaviours that serve to maintain SAD and lead to the increased distress reported by those with SAD following social engagement. While this study found SAD modulated several aspects of the subjective experience of acute social stress in daily life, these effects did not occur for the daily physiological acute stress response. Specifically, participants in the SAD group did not differ from healthy adults during any phase of the study on any of the diurnal cortisol measures. Surprisingly, higher levels of average cortisol were observed in both SAD and healthy controls during the baseline phase.

4.1 Subjective Daily Experiences

To our knowledge, no study has examined the affect and self-esteem experience of SAD in daily life and the associations between a laboratory based social stressor and the subjective and physiological responses to this social stressor in the broader daily life context. Our finding of higher levels of negative affect in those with SAD at baseline and across the week of sampling compared to healthy controls is consistent with other EMA findings of negative affect in SAD (Farmer & Kashdan,

2012; Farmer & Kashdan, 2014). In SAD, despite the large focus on the known negative bias in the disorder, deficits in positive affect and experiences have also been established in the disorder (Kashdan, Weeks, & Savostyanova, 2011). Our finding of lower happiness overall aligns with existing research of diminished positive affect and experience in SAD (Farmer & Kashdan, 2014; Kashdan et al., 2013). This supports the continued importance for examining the positive in SAD, not just the negative. This study demonstrated that the overall subjective experience in SAD varied according to engagement with acute social stress, with the overall diminished positive and higher negative affective experience observed in SAD exacerbated during the anticipation of and recovery from social engagement. These results further suggest that SAD individuals were more reactive to social stress compared to healthy controls, observed in the increased anxiety and anger and decreased in happiness and appearance satisfaction in those with SAD during the recovery phase. The increases in anxiety and decreases in happiness and appearance satisfaction in the SAD group during anticipation, compared to healthy controls, suggests the SAD group were also more reactive to the anticipation of social engagement.

State self-esteem and fluctuations in self-esteem have not been extensively examined in SAD, with only one EMA study examining changes in self-esteem in daily life (Farmer & Kashdan, 2014). Non-SAD based EMA studies of self-esteem have demonstrated fluctuation in self-esteem as a predictor of vulnerability for depression (Kernis et al., 1998), higher levels of stress reactivity (Greenier et al., 1999) and impairments in well-being (Kashdan, Uswatte, Steger, & Julian, 2006). With varying self-esteem shown to impact mood, stress reactivity and overall wellbeing outside of SAD, it is important to understand how changes in self-esteem in the disorder could contribute to the overall symptomology of the disorder and maintenance of behaviours associated with SAD (e.g. avoidance). Interestingly,

results from this study demonstrate SAD participants had lower levels of self-esteem in performance, appearance and social facets of self-esteem at baseline and overall, but only appearance satisfaction varied (i.e. reduced further compared to baseline) in SAD in the lead up to and recovery from a social task. This finding of relatively stable measures of state self-esteem across the phases is contrary to Farmer and Kashdan (2014) who found greater instability in self-esteem and higher likelihood of acute changes in self-esteem in SAD compared to healthy controls. Our study however, specifically looked at phase effects, as opposed to varying self-esteem from one assessment to the next across the seven days of EMA. As such, the results from our current study reflect mean phase levels of self-esteem and not moment-to-moment fluctuations. Across the phases in this study no effects for performance or social based self-esteem for SAD were observed, demonstrating that performance and social self-esteem were relatively stable in the anticipation of and response to acute social stress. Appearance based state self-esteem did vary significantly in the anticipation of and recovery from acute social stress in SAD, though the changes were small. These results demonstrate that while individuals with SAD report consistently lower levels of self-esteem than healthy controls, state self-esteem remained relatively consistent across the protocol despite the experience of an acute social stressor.

We found that those with SAD reported higher threat awareness, along with less environment security at baseline and across the entire protocol when compared to healthy adults. These results demonstrate that known hypervigilance toward fear of negative evaluation, judgement and scrutiny and the lack of security known to SAD is pervasive and consistent across time. Surprisingly, the SAD group did not demonstrate significant changes in these measures in anticipation of, or recovery from an acute social stressor when compared to baseline, lending support to the notion that

the awareness of judgement and scrutiny from others and a lack of security in SAD is persistent, despite time and context variations.

4.2 Physiological Daily Experiences

The daily life physiological findings from this study are consistent with studies investigating short-term responding to an acute social stressor in the lab, which have not consistently found different patterns of physiological responding among individuals with SAD compared with healthy controls (Jamieson et al., 2013; Klumbies et al., 2014; see Chapter 6 also). These results demonstrate the physiological response to acute stress in SAD is comparative to healthy controls. When looking at these results in relation to the anxiety disorder umbrella, these results support theories of SAD that suggest the disorder does not involve the extreme form of focused fear or threat reactivity observed in specific phobias, nor do people with SAD demonstrate chronic overloading of the HPA axis system similar to individuals with GAD and panic disorder (see Lang & McTeague, 2009). Physiologically, the HPA axis activity in daily life in SAD does not differ from the expected normal experience.

Only one statistically significant difference in salivary cortisol was found in our examination of study phases, with higher average salivary cortisol levels found in the baseline phase across all participants (i.e., among both SAD and healthy controls). Several explanations for this finding are possible. Firstly, these elevated cortisol concentrations may have been as a consequence of the novelty of the project to participants. It is possible the elevated cortisol was potentially due to unfamiliarity with sampling, with higher average levels representative of the newness of the sampling procedure, and the demand of having to meet the requirements of the timing

schedule and further, having to do so correctly. It is also possible participants did not make note of potential influences on salivary cortisol in their record book, with human error (e.g. drinking or eating right before a sample, sampling in close proximity to intense exercise, smoking) not accounted for in individual analyses. We note existing research of subjective reporting by participants has found the presence of an elevation bias in subjective reports, with experimental investigations finding an initial elevation in reports that was later followed by a decline, suggesting that the process of beginning a study causes an initial elevation bias that will eventually decline (Shrout et al., 2018). Another possible explanation for the observed increase in baseline could be similar to the phenomenon of whitecoat hypertension, in which a patient's blood pressure is higher when taken in a medical setting than it is in other settings, such as home (Mancia, Grassi, Parati & Zanchetti, 2015). Given the proximity of the baseline sampling to the initial laboratory session, we may have observed a similar elevation in cortisol as a consequence of the clinical setting the initial lab was conducted in.

4.3 Implications

The combination of the use of EMA techniques to assess the subjective and physiological daily life experience in the lead up to and recovery from a laboratory based acute social stressor, in a group of participants with SAD and healthy controls, provides a unique and particularly rich data set. This data provides insights into how the experience of SAD unfolds in daily life, outside of the lab context. The methodology allows for capture of fluctuations in affect, self-esteem, threat and environment security in response to acute social stress that may otherwise have been missed if limited to the immediate context of the laboratory session. We found that while physiologically the two groups were comparative, the subjective experience

reported by those with SAD was significantly different to healthy controls. Specifically, we found that not only did those with SAD significantly differ from healthy controls across all three phases consistently in all subjective measures (see Table 3 and 4, and figure 4) but the SAD group also significantly differed in some subjective outcomes according to the anticipation of and recovery from acute stress. That is, greater reactivity to social stress was observed in SAD by the increased anxiety and anger, and decreased happiness and appearance satisfaction in the anticipation and recovery phases (increased anger in recovery only) when compared to baseline and healthy controls. This reactivity in negative affect is in line with the Farmer and Kashdan (2014) finding of greater instability in negative affect in SAD, however contrary to their finding of relatively stable positive affect, we found those with SAD demonstrated reactivity in measures of both negative affect (i.e. anxiety and anger) and positive affect (i.e. happiness) in response to acute social stress.

These changes in subjective reports across phases by those with SAD demonstrates that the subjective experience of acute social stress was not contained to the immediate social context, instead the negative impact persisted across time, on a number of subjective measures, in the anticipation of and recovery from social engagement. This suggests the experience of social engagement was more intrusive in those with SAD compared to healthy controls, with the SAD group more reactive to the social engagement. Understanding the experience of this disorder in consideration of context and time-dependent fluctuation lends support toward current clinical initiatives in mental health that focus on the underlying mechanisms of dysfunction in mental health disorders and how these dysfunctional processes unfold, as opposed to the strict boundaries of diagnostic categories and symptoms (Insel et al., 2010). Through the use of EMA methods, this study captured the daily life experience of affect, self-esteem, threat awareness and environment security to examine the varying

profile and fluctuations of these subjective emotional experiences in SAD, alongside physiological sampling. The resulting data contributes to the understanding of how those with SAD respond, both in the mind and the body, to acute social stress in daily life. These results add to the understanding of how the anticipation of and consequences of social engagement is experienced in daily life and how fluctuations in these psychological states occur around social stress in SAD. Specifically, while physiologically comparative, individuals with SAD did experience higher anxiety, less happiness and less satisfaction with their appearance in the lead up to a social stress task, and further, in the days following completion of the social stress task those with SAD had higher anxiety and anger, and reported less happiness and appearance satisfaction. This study contributes to the understand that the maladaptive subjective experience of social engagement in SAD is not isolated to the social context, and that the consequences of social engagement extend beyond the immediate engagement and across daily life.

This study expands laboratory based research that has reported a separation between the subjective and physiological experience of acute social stress in SAD. We found that the presence of no physiological, but significant subjective differences in SAD compared to healthy adults that has been observed in the laboratory (e.g. Study 1, Chapter 6), is also apparent in daily life. From this we can understand that in SAD, the body (specifically the peripheral cortisol response) responds the same as healthy adults when faced with social stress, but the psychological experience between those with the disorder and typically healthy adults varies largely. This understanding of how those with SAD report their experience is particularly important to clinical psychology, and replicates similar results from other social anxiety studies that demonstrated despite exaggerated or increased reporting of the subjective experience of stress, those with social anxiety demonstrate relatively comparative

physiological arousal in response to stress (Jamieson et al., 2013; Krämer et al., 2012; Mauss, Wilhelm, & Gross, 2004). In this study specifically, the social stress task was the same for the entire population, however, when compared to healthy controls, the subjective reports by those with SAD demonstrated significantly higher levels of impact in the anticipation of the stressor (i.e. higher anxiety, lower happiness, less appearance satisfaction) and following the stressor, seen in the higher negative affect (i.e. anxiety and anger), lower positive affect (i.e. happiness) and appearance satisfaction during recovery. In this study, psychologically, the experience of the same social encounter was overestimated (i.e. the magnitude of subjective reporting) in SAD compared to healthy controls. This potentially supports evidence of a disjunction between the physiological and subjective experience of stress in SAD, with those with the disorder reporting the intensity and impact of the experience to a far higher degree than healthy adults, despite physiologically being comparative. This study contributes to the lab-based literature by suggesting these differences in subjective reporting of stress in SAD extend beyond the immediate context of the social stressor across days, including the anticipation of and recovery from social tasks.

4.4 Limitations

Our findings must be considered in light of several limitations. Firstly, this study relied heavily on participants to independently and accurately sample both their subjective and physiological experience. We relied on participants to not only remember the nuances of the protocol and sampling instructions, but further to be honest and transparent in regard to their reporting (both accuracy of surveys and at-home sampling of saliva). While we aimed to reduce false reporting (i.e. removal of surveys with too quick a reaction time, or saliva samples outside of typical bounds of cortisol concentration) we must acknowledge there are limitations to how strictly this

can be controlled in daily life sampling. Further, it would be of interest for future studies to align phone surveys to physiological sampling, to examine these directly in relation to one another and determine if there are associations between the subjective and physiological daily life experiences, such as the concordance observed between anxiety and salivary cortisol in Study 1 (Chapter 6).

We also acknowledge the limitations that may arise from this form of ambulatory assessment, particularly in our use of a custom smartphone phone application on participants own smartphone. Participants required a thorough introduction to and training for the use of the SEMA app, increasing the time demand of participants and further, there was an additional time commitment for the researcher to monitor missed surveys, participant compliance and overall progress of the EMA protocol. Technological difficulties, such as delays in the commencement of surveys (i.e. problems with syncing with participants phone when set to 'active'), and problems with notifications on the android (vs. iOS) interface were also present in this study. We note the increased participant burden and technological difficulties experienced inevitably contributed to the varying compliance of participants, and differences in the number of completed surveys (particularly within phases) across the entire participant group. These examples speak to the importance of large sample sizes in EMA data due to the potential exclusion of a number of problematic responses or missed surveys during the data cleaning process.

In light of our findings demonstrating 'phase' effects, both in the subjective sampling for the SAD group and baseline salivary cortisol across groups, it would be valuable for future studies to implement more controlled phase division along with careful management of the notification of the social task. The current study demonstrated variability in the length of time and number of surveys completed during each phase. The length of the baseline phase varied according to the time Lab

1 was completed by the participant, with SEMA surveys starting post Lab 1. The anticipation phase was shorter in length compared to the baseline and recovery phases, producing fewer observations. Lastly, the recovery phase was the longest of all three phases and produced the highest average number of observations. Future research may consider methods that capture a 'true' baseline phase (without interference from Lab 1, e.g. to avoid the whitecoat phenomenon) and methods in which there is equal assessment within the three sampling phases (e.g. the length of the anticipation phase is equal to baseline and recovery).

4.5 Conclusion

Our research contributes new insights into the phenomenology of SAD through the use of a multimodal daily life and laboratory based approach that explored changes in the subjective and physiological responses to a social stressor in SAD. Specifically, we found that compared to their everyday experience (i.e. baseline assessment), individuals with SAD demonstrated heightened anxiety, reduced happiness and lower appearance satisfaction during the anticipation of a social stressor. Following the social stressor, not only did individuals with SAD continue to report heightened anxiety, less happiness and appearance satisfaction, but they also reported higher levels of anger compared with healthy controls. Interestingly, while other measures of negative affect (e.g. sadness and embarrassment), state self-esteem (e.g. self-efficacy and fear of negative evaluation), threat awareness and environment security were significantly different in SAD compared to healthy controls in general, these measures did not significantly vary across the phases of the study. While these findings require future replication, they suggest that some SAD symptoms (e.g.

happiness or anxiety) may be more context-dependent or likely to fluctuate across time than others (e.g. sadness), which may be more persistent experiences.

This study contributes to a small amount of EMA literature examining the daily experience of individuals with SAD by demonstrating while some measures of affect (e.g. anxiety and happiness) and self-esteem (e.g. appearance satisfaction) were likely to vary according to engagement with a social stressor, others surprisingly remained consistent regardless of acute social stress (e.g. no higher fear of negative evaluation, no loss of self-efficacy, no higher embarrassment following acute social stress). We add to support to existing laboratory based research that has demonstrated large subjective, but not physiological, differences in response to social stress in SAD, when compared to healthy adults (e.g. Jamieson et al., 2013; Klumbies et al., 2014; Mauss et al., 2004). Specifically, individuals with SAD did not demonstrate any physiological salivary cortisol differences either at baseline or in response to acute social stress, compared with age- and sex-matched healthy adults. This study emphasises that in the absence of any physiological differences in SAD (specifically, peripheral salivary cortisol) models of SAD that focus on the cognitive and psychological experience of the disorder are highly applicable, such as the IAM model of SAD (Wong & Rapee, 2016). By using a multimodal approach, with assessment of subjective, physiological, state and trait, and clinical characteristics in SAD in both daily life and lab, we demonstrate a novel way to combine laboratory-based and ambulatory assessment methods to garner a richer, more in-depth understanding of mental disorders, capitalising on both the high internal validity of laboratory based methods and the high ecological validity of daily life sampling.

CHAPTER 8. General Discussion

8.1 General Overview and Summary of Findings

The primary goal of this thesis was to examine the subjective and physiological social stress response in Social Anxiety Disorder (SAD) across daily life. The disorder is characterised by fear or anxiety around potential judgement, scrutiny and negative evaluation by others in social situations, and has been linked to cognitive, neurological, physiological, psychological and behavioural dysfunction (see Chapter 2). It is important to understand how individuals with SAD respond to stress, specifically social stress, if we are to improve the psychopathological and health outcomes of those affected. Much of the existing understanding of SAD comes from research in the laboratory setting, with little known about the disorder in daily life. Given that symptoms of SAD are context-dependent and fluctuate over time, it is difficult to assess behaviours and experiences realistically in the laboratory or by means of retrospective reporting. This thesis employed EMA techniques to deepen our understanding of the acute social stress response of those with SAD through frequent assessments in their natural environment (see Chapter 3). In order to examine the acute social stress response in daily life in SAD, it was imperative to the structure of the methodology that all participants incurred a social stress task that induced a similar acute stress response. While not a core aim of the thesis, it became apparent when seeking to implement the widely used Trier Social Stress Test (TSST; Kirschbaum et al., 1993) as our laboratory based acute stress task that there was no detailed protocol available, which lead to the development and valuable contribution to the literature of a detailed step-by-step introductory guide to ease the application of implementing the TSST (see published manuscript in Chapter 5). The unique and novel contribution of this thesis was the examination of the acute social stress response, initiated via a lab-based acute social stress protocol, in SAD across daily life using EMA assessment techniques. At the core of this thesis are two empirical studies.

The first study examined the efficacy of the acute social stress task in the current participant sample (Chapter 6). The second and primary study of this thesis examined this acute stress response in daily life (Chapter 7). Next, a brief summary of each of the studies are presented for ease of remembering.

In Study 1 (Chapter 6), we examined the acute stress response in the lab to (i) confirm that the expected stress response occurred in all participants, and (ii) compare the SAD acute stress response to healthy controls. Physiologically, no group differences in salivary cortisol concentration at baseline or over the course (in both total growth and reactivity) of the stress protocol were observed between SAD and healthy controls. A small group of individuals (21%) did not demonstrate the expected salivary cortisol response, i.e. cortisol non-responders. Subjectively, those with SAD reported significantly higher anxiety, sadness, tiredness, and withdrawal, and lower levels of happiness compared to healthy controls at baseline and in growth across the stress protocol. Moreover, those with SAD experience changes in anxiety and sadness at a greater intensity compared to healthy controls across the stress task. We provide evidence of concordance (i.e. a moderate positive association) between subjective anxiety and salivary cortisol in response to stress when statistically accounting for temporal dynamics in the analyses, though this did not differ between SAD and healthy controls. This lab-based study concluded that, despite large subjective experience differences to acute social stress, those with SAD have similar hypothalamic-pituitary-adrenal (HPA) axis activity compared to healthy controls, with evidence for concordance between the response systems.

In *Study 2* (Chapter 7)*,* the daily life subjective and physiological response to acute stress was observed during three phases: (i) baseline, across the (ii) anticipation of and (iii) recovery from the TSST. Subjective reports were gathered via the EMA smartphone application SEMA2 (Harrison et al., 2017), and physiologically, the

diurnal cortisol rhythm was captured via three days (once during each phase) of at-home saliva sampling. Overall, those with SAD demonstrated higher levels of negative affect, lower levels of positive affect, lower self-esteem, increased threat awareness and less environmental security compared to healthy controls. During the anticipation of and recovery from the social stressor, SAD individuals demonstrated heightened anxiety and reduced happiness as expected, but also reduced appearance satisfaction. When recovering, SAD individuals additionally demonstrated greater anger reactivity. Neuroticism and conscientiousness moderated the subjective experience of the entire population, and sex differences in subjective report were apparent at the within-group (e.g. SAD males and female differences in anger) but not between-group level. No diurnal salivary cortisol differences were found between SAD and healthy adults across any of the three phases, although higher overall across the day cortisol was found during the baseline phase for both groups (compared to the anticipation and recovery phases). This study demonstrated that while the HPA axis activity in response to stress of those with SAD is comparative to healthy adults, the dysfunction in the subjective experience known to SAD was pervasive across time, with some subjective measures also exacerbated (i.e. increased from baseline) during the anticipation of and recovery from social stress.

Overall, this thesis provides evidence that those with SAD (compared to healthy controls) have a typical physiological salivary cortisol response to social stress, evident both in the immediate laboratory task (Study 1) and in daily life (Study 2). This was in contrast to the large differences between groups in subjective experiences, as expected. This thesis, especially study 2, also reports on additional psychological functions implicated in SAD and in response to social stress (self-esteem, appearance satisfaction, anger). Next we will discuss the implications and contributions of the findings from this thesis, the strength of this thesis including how

daily life methodologies offer an ecologically valid way to explore a context-dependent disorder where symptoms fluctuate across time, as well as general limitations of this research and future directions, before concluding with final remarks.

8.2 Implications and Contributions of This Thesis

The following section will discuss key findings from this thesis and highlight the implications and contributions of this thesis to the SAD literature. We discuss evidence for separateness between the psychological, cognitive and autonomic responses to stress from the hypothalamic-pituitary-adrenal (HPA) axis in SAD. We will also examine how this thesis add supports to theoretical models of SAD and how this thesis fits within the larger discussion around classification and treatment of SAD. Lastly, we will discuss encouraging adaptive psychological appraisals (e.g. arousal re-appraisal) of the 'normal' stress response in the treatment of the disorder.

The key finding of this thesis is the support for significantly heightened subjective stress responses in SAD, but no physiological differences in one of the primary stress response systems, the HPA axis. Collectively, findings from the two empirical studies suggest that the clinical impairment and dysfunctional mechanisms of SAD are central to cognitive psychological processes (i.e., brain and central nervous system), with the peripheral HPA axis (i.e. salivary cortisol) system showing a comparative profile between SAD and healthy controls. The thesis supports existing SAD literature demonstrating consistently impaired affect (i.e. higher negative affect, lower positive affect), lower self-esteem and heightened threat awareness in SAD across daily life, when compared to healthy controls. The unique element of this study is the contribution we make to the literature by inserting a reliable and valid lab-based acute social stressor within our protocol, to ensure all participants engaged with acute

social stress during their participation. This allowed us to circumvent possible avoidance behaviours frequently observed in SAD, and to examine directly the impact of social engagement across daily life in the disorder. Through this methodology, we contribute novel insights to the understanding of SAD by demonstrating that the impact of acute social stress was not isolated to the immediate context of the social experience (e.g. in the lab only), instead greater reactivity was observed in a number of subjective consequences of the social stressor (i.e. increased anxiety and anger, reduced happiness and appearance satisfaction) during the anticipation of and recovery from social stress. The demonstration of increased reactivity in response to social stress in daily life in positive affect (i.e. happiness) was a new contribution to the EMA and SAD literature, with previous literature finding negative affect instability but not positive affect instability (Farmer & Kashdan, 2014). We also contribute an interesting addition to the understanding of state self-esteem in daily life in SAD. Specifically, the increased social (i.e. fear of negative evaluation), lower performance (i.e. self-efficacy) and lower appearance (i.e. satisfaction with) state self-esteem measures observed for SAD were all significantly and consistently different compared to healthy controls across daily life. This was in line with existing trait based assessment of self-esteem in SAD that demonstrated self-esteem, self-efficacy and self-criticism were negatively correlated with social anxiety, such that as rating of social anxiety increased, self-esteem, self-efficacy and self-criticism decreased (Iancu, Bodner, & Ben-Zion, 2015). Interestingly, in our study only one of these measures (i.e. appearance satisfaction) varied according to the social stress encounter and the phases of participation. This suggests that some measures of state self-esteem in SAD in daily life (i.e. self-efficacy and fear of negative evaluation) are consistent across time and do not vary significantly, despite participants engaging in a performance driven and social-evaluative social stress encounter. This was in line with previous

EMA research in SAD examining self-esteem, which reported that instability in self-esteem across daily life was driven by average self-esteem level differences (Farmer & Kashdan, 2014). We provide support toward a pervasive negativity bias, heightened stress sensitivity (i.e. reactivity to social stress), maladaptive post-event processing following social engagement and overall affective impairment and diminished self-esteem in SAD, when compared to healthy adults.

8.2.1 Differences in psychological, central and autonomic stress responses in SAD, but not HPA axis activity. In this thesis, consistently across baseline and in responses to acute social stress no physiological differences in salivary cortisol were found between individuals with SAD and healthy controls. This largely contrasts the significant differences that were observed in the subjective reports of those with SAD compared to healthy controls. For reference, the typical human stress response via the central and autonomic nervous systems is presented in Figure 8.1. The two blue boxes summarise evidence from this thesis and current evidence for the stress response in SAD is presented in the green boxes.

The *stress system* integrates various brain structures that collectively detect, interpret and process stressful stimuli. Different stress stimuli engage different brain networks or localised regions, with the stressor stimulating rapid activation of two key components of the human stress response, the HPA axis and the autonomic response via the Sympathetic-Adrenal-Medullary (SAM) axis (Rotenberg & McGrath, 2016). While there is overlap in the processes of these two complementary axes, the slow-acting HPA axis response to stress (endocrine response) and the fast-acting SAM response to stress (via synaptic transmission) represent two distinct aspects of the stress response (Bitsika, Sharpley, Sweeney, & McFarlane, 2014).

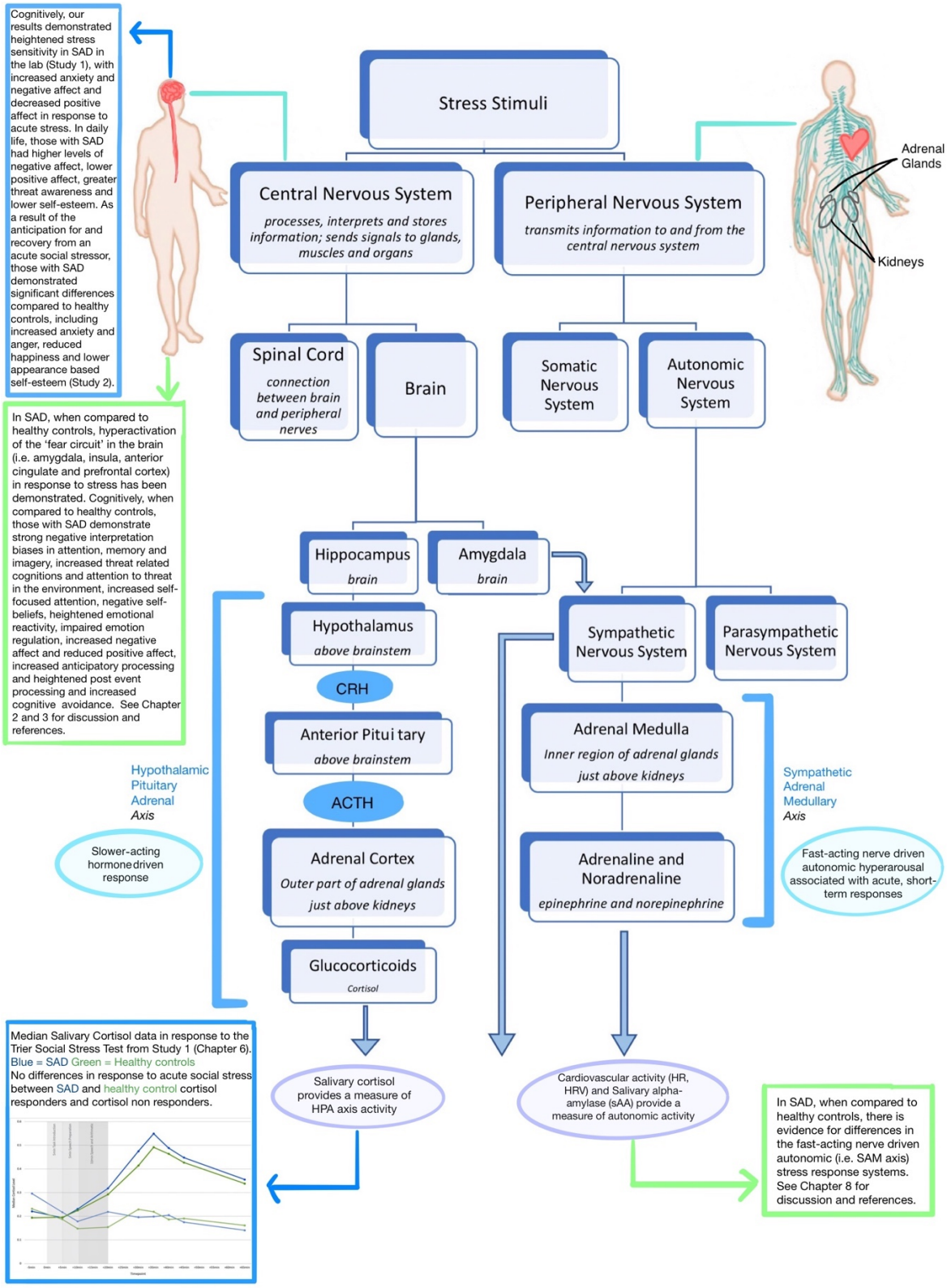


Figure 8.1. Pathways of the human stress response, highlighting differences and similarities in the stress response observed between those with Social Anxiety Disorder (SAD) compared to healthy controls in this thesis (light blue text boxes) and exiting literature (green text boxes). Supporting literature cited in text. Note. CRH – corticotropin releasing hormone, ACTH - adrenocorticotropic hormone, HPA – hypothalamic-pituitary-adrenal, SAM – sympathetic-adrenal-medullary

The complex phenomenon of the stress response is not restricted to neuroanatomy or activation of these two inter-related HPA and SAM stress systems. The stress response is also influenced by the type, length, frequency and intensity of the stress experience and the short and long term consequences of the stress response in the body (Godoy, Rossignoli, Delfino-Pereira, Garcia-Cairasco, & de Lima Umeoka, 2018). The identification of the activation of the different stress networks and how these stress networks respond differently in SAD compared to healthy controls is critical to understanding the psychological and physiological impact of stress on those with SAD, the implications of these differences on the psychopathology of the disorder, and the understanding of stress and its health associations in SAD (Rotenberg & McGrath, 2016).

The fast-acting autonomic nervous system (ANS) initiates physiological changes in the body in response to a stressor through synaptic transmissions across the two branches of the ANS, the sympathetic and parasympathetic nervous system. The parasympathetic nervous systems has inhibitory effects over the sympathetic nervous system, which are withdrawn in response to stress to facilitate the sympathetic nervous system activating hyperarousal in the body, known as the fight or flight response (Porges, 2007). This in turn activates the SAM axis which produces adrenaline and noradrenaline and measurable changes in cardiovascular functioning (Murison, 2016). Conversely to the fast-acting ANS system, the HPA axis response to stress is a hormone driven system and thereby slower-acting. The central nervous system and endocrine response are tightly interconnected to initiate the HPA axis response to stress (Ulrich-Lai & Herman, 2009). Release of the corticotrophin-releasing hormone from the hypothalamus stimulates the HPA axis response, acting on the pituitary gland that stimulates the adrenal cortex and culminates in the release of glucocorticoids, such as cortisol (Rotenberg & McGrath, 2016). Cortisol

contributes to increases in ANS activity, and is said to enhance the sympathetic cardiovascular stress response seen in increased heart rate (Rotenberg & McGrath, 2016), demonstrating interrelatedness between the two systems. HPA axis activity can be assessed via salivary cortisol (Bozovic, Racic, & Ivkovic, 2013). Autonomic SAM axis activity can be assessed via salivary alpha-amylase (sAA) concentration, with sAA directly related to norepinephrine activity and varies in relation to the stress markers of norepinephrine, cardiac activity and electrodermal activity and is also associated with state anxiety (Bitsika et al., 2014; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). Autonomic activity can also be directly assessed via heart rate and heart rate variability. Existing SAD research in adults has found varied results for salivary cortisol, sAA, and cardio regulatory differences. Elevated sAA (as an indicator of SAM axis differences) has been demonstrated in SAD when compared to healthy controls in basal non-stress conditions (Van Veen et al., 2008) but not in response to acute stress (Klumbies et al., 2014). In response to physical stress (e.g. electrical stimulation), elevated sAA but not salivary cortisol was found in SAD compared to healthy controls (Tamura et al., 2013). In SAD, when compared to controls, some studies have demonstrated no differences in salivary cortisol in response to acute stress (see Klumbies et al., 2014; Krämer et al., 2012; Martel et al., 1999), while others have found those with SAD having significantly higher cortisol reactivity relative to the controls (see Beaton et al., 2006; Condren et al., 2002; Furlan et al., 2001; Roelofs et al., 2009; van West et al., 2008). Though this research did not analyse cardiovascular functioning, altered cardio-autonomic regulation has been clearly demonstrated in SAD, specifically, reduced heart rate variability at rest and in response to stress when compared to controls (see Alvares et al., 2013; Gaebler et al., 2013; Pittig et al., 2013).

One alternative explanation for our finding of comparative HPA axis activity in response to acute social stress between SAD and healthy controls is possible habituation of the HPA axis having occurred across time in SAD, as a consequence of repeated activation of the HPA axis from exposure to social stress. It is proposed that lower cortisol concentrations may reflect coping and adaptation as a consequence of repeated stress (Fries, Hesse, Hellhammer, & Hellhammer, 2005), with reduced cortisol reactivity observed in other stress-related disorders (e.g. PTSD; Heim et al., 2000). It is argued that hypoactive cortisol responding (i.e. decreased responding) could also occur as a consequence of traumatic experiences in early childhood (Gunnar & Vazquez, 2001). In trait shyness or non-clinical social anxiety samples, it is argued that a pattern of low salivary cortisol levels may reflect changes in the adrenocortical stress response system as a consequence of long-term management of shyness and social anxiety which leads to high allostatic loading (Fries et al., 2005) and in order to cope with high allostatic loading and a dysregulated adrenocortical system, hypo-responsivity in the HPA axis may occur (Fries et al., 2005). To explore this alternative explanation, the HPA axis activity of children and adolescents with SAD in response to stress and whether this is comparative to adults with the disorder was considered. Evidence from child and adolescent studies has also demonstrated mixed results regarding autonomic, endocrine and perceived stress responses. In children, associations between high social anxiety and elevated sAA, but not salivary cortisol, in response to stress have been reported (Payne, Hibel, Granger, Tsao, & Zeltzer, 2014) and these results mirror findings of elevated sAA in adults (Van Veen et al., 2008). When compared to healthy controls, children diagnosed with SAD did not differ in salivary cortisol, sAA or heart rate in response to the child-version of the TSST (Krämer et al., 2012), however, children with SAD did demonstrated significantly elevated heart rate across the entire assessment period (suggesting

altered cardiovascular functioning). In adolescents with SAD, no significant differences in salivary cortisol in adolescent females diagnosed with SAD following acute social stress have been reported (Martel et al., 1999). Review of child and adolescent salivary cortisol, sAA and cardio function suggests that the alternative explanation of possible habituation of the HPA axis is unfounded, with no differences in HPA axis activity in response to stress in SAD also observed across the child and adolescent population.

Combined, existing HPA axis activity evidence in SAD, our evidence for no altered HPA axis activity in SAD, existing evidence of autonomic SAM system hyperactivity in SAD, and neurological evidence for central nervous system (CNS) hyperactivity in SAD (e.g. amygdala activation in response to social threat; Evans et al., 2008; Goldin et al., 2009) suggests a possible disconnect between the fast-acting central nervous system and autonomic systems and the slower-acting endocrine HPA axis stress response systems in SAD. It is interesting to explore the existing evidence for autonomic differences in the form of cardio dysregulation in SAD, in light of our evidence of no HPA axis differences and what ANS functioning represents in a social disorder. Specifically, differences in ANS functioning in SAD are highlighted in biobehavioural models such as Polyvagal theory. Polyvagal theory directly links social interaction with ANS functioning, with the ANS said to modulate the affective experience and social behaviour of individuals (Porges, 1997). This theory suggests the vagus nerve (in the parasympathetic nervous system) promotes engagement or disengagement with the social environment, and parasympathetic cardiac control inhibits sympathetic activity to promote regulation of emotion and prosocial behaviour (Alvares et al., 2013). Heart rate variability is an index of ANS activity and optimal heart health is reflected in increased heart rate variability (Thayer, Yamamoto, & Brosschot, 2010). As such, Polyvagal theory suggests disordered social

functioning and ability for social engagement will be reflected in decreased ANS regulation, suggesting in SAD we would expect to observe reductions in ANS cardio regulation, with the reduced cardio control potentially associated with social fear, avoidance and inhibition (Alvares et al., 2013).

In sum, evidence from this thesis supports that SAD does not display the differences in the common stress marker of HPA axis responding that a more diffuse fear or threat disorder does (e.g. overloading of the HPA axis seen in GAD or PTSD). This thesis supports that the subjective response does differ largely from the typically healthy adaptive experience. Combined with existing evidence of impaired ANS regulation and central nervous system hyperactivity in SAD, this thesis lends support to dysregulation in the central and autonomic, but not the peripheral HPA axis, stress response systems. We support two understandings of stress in SAD in this thesis, firstly that physiological differences in SAD are observed in some stress response systems linked to the prosocial domain (i.e. ANS) but not observed in all physiological stress response systems (i.e. HPA axis), and second, that the dysfunctional and maladaptive mechanisms contributing to the subjective distress experienced in SAD play out largely in the central cognitive and psychological domains.

8.2.2 Theoretical models of SAD. The human stress response is a complex phenomenon, with several distinctive cognitive, emotional, physiological and behavioural systems known to be involved in the stress response (Campbell & Ehlert, 2012). The current understanding of these systems is that there is a lack of synchrony in these responses, with each response representative of several independent and varying components, as opposed to previous understanding of the human stress response being a single, synchronised system (Campbell & Ehlert, 2012). Evidence from this thesis overwhelmingly supports a lack of synchrony in the subjective

psychological and physiological (HPA axis) experience of social stress in SAD, with individuals with SAD physiologically comparative to healthy adults in both the lab and daily life, but vastly different in their subjective experience. The subjective experience of social-evaluative threat in SAD was of particular interest to this thesis. While the stress response has been widely studied, this is often in reference to physiological stress reactivity and responding, often without accounting for the psychological response to stress (Campbell & Ehlert, 2012). The complex emotional and cognitive responses to stress are deserving of as much attention as the physiological stress response has incurred, particularly as emotional and cognitive processes are considered to be the primary pathways through which arousal can activate maladaptive physiological states and consequently lead to long-term psychopathology (Campbell & Ehlert, 2012).

It is widely understood that individuals vary in the amplitude, threshold, peak and overall style of affective experience (Davidson, 2000), including the awareness they have of this affective response (Mauss et al., 2005). Two key facets of emotional responding include stress reappraisal (i.e. cognitive appraisal aiming to re-evaluate stimuli to reduce the impact and prevent the occurrence of negative affect) and emotion regulation (i.e. downregulation of current and ongoing affective states)(Campbell & Ehlert, 2012). The subjective emotional experience of stress in SAD is typically as a result of appraisal of social stimuli as harmful or threatening. Our research demonstrates support for impaired stress reappraisal and emotion regulation in SAD in response to social stress, both in the acute and daily life setting. This is implied by the observation of overall heightened negative affect, diminished positive affect, lower self-esteem and higher threat awareness observed in SAD. We also provide evidence for those with SAD exhibiting heightened stress sensitivity, with significantly stronger subjective reactions to a socially stressful experience

compared to controls. Research has demonstrated associations between level of stress sensitivity and risk for development and maintenance of mental health disorders (Mezulis, Funasaki, Charbonneau, & Hyde, 2010; Morris, Rao, & Garber, 2012). Existing EMA research into stress sensitivity in SAD has demonstrated those with the disorder display greater stress sensitivity when compared to healthy controls, particularly in level of negative affect in response to a socially stressful event (Farmer & Kashdan, 2015). Our study extends this finding by directly observing stress sensitivity in both the immediate context of social stress (i.e. during an acute social stressor) and the broader consequences of stress in daily life. Those with SAD displayed heightened subjective stress sensitivity during the acute stressor, reporting significantly higher anxiety and sadness and less happiness than controls in response to acute stress. This heightened stress sensitivity was pervasive, with the SAD group continuing to report higher negative affect (e.g. anger and anxiety) and lower positive affect (e.g. happiness) in the days following the social stressor. Individuals with SAD also demonstrated their appearance based state self-esteem was impacted by social stress, with appearance satisfaction significantly lower in the anticipation of and recovery from social stress, compared to baseline. The state self-esteem measures of self-efficacy and fear of negative evaluation while lower and higher (respectively) overall, these measures surprisingly did not vary as a consequence of social engagement and remained consistent across the phases of this study. This suggests some measures of state self-esteem are more stable than others in response to social stress in SAD. Further, we found evidence that personality factors such as neuroticism and conscientiousness influence subjective ratings, supporting the role of personality in the aetiology of SAD.

In light of the above evidence, this thesis supports models of SAD that highlight the role of the cognitive, neurological, psychological and behavioural factors

in the aetiology and maintenance of SAD, specifically cognitive (Clark & Wells, 1995; Heimberg et al., 2014; Hofmann, 2007; Rapee & Heimberg, 1997) and emerging models of SAD, such as the integrated aetiological and maintenance (IAM) model of SAD (Wong & Rapee, 2016). If we examine the central aetiological and maintenance factors of SAD theorised in the IAM model for SAD (see Figure 8.2; Wong & Rapee, 2016), our research along with existing evidence lends support to the role of inherited tendencies (i.e. personality traits such as neuroticism), greater evaluation of social stimuli as threatening (e.g. heightened anxiety, higher negative affect and lower positive affect around social engagement), negative social evaluative cognitions (e.g. heightened stress sensitivity and interpretation of social stressors as negative), neurobiological changes (e.g. heightened activation of the fear circuitry in the brain), primary cognitive processes (e.g. greater self-focused attention and threat awareness) and secondary processes (i.e. anticipatory processing and post-event processing) in the moment-to-moment experience of anxiety in daily life and the subsequent impairment that is experienced in SAD.

While our research adds support to the existing models, future research will need to continue to test and validate new emerging models such as the IAM model, including other aetiological factors (e.g. specific life events or peer experiences) and behavioural processes (e.g. avoidance and escape), which were beyond the scope of our study. While our study is the first to examine elements of the IAM model of SAD using daily life assessment and directly in relation to engagement with social stress, specific validation of the IAM model is still required. Future SAD research using EMA techniques may directly examine key associations between the specific aetiological factors of the model to the daily life experience of SAD and further test how the suggested maintenance factors of SAD unfold in daily life in those with SAD, particularly in relation to social encounters.

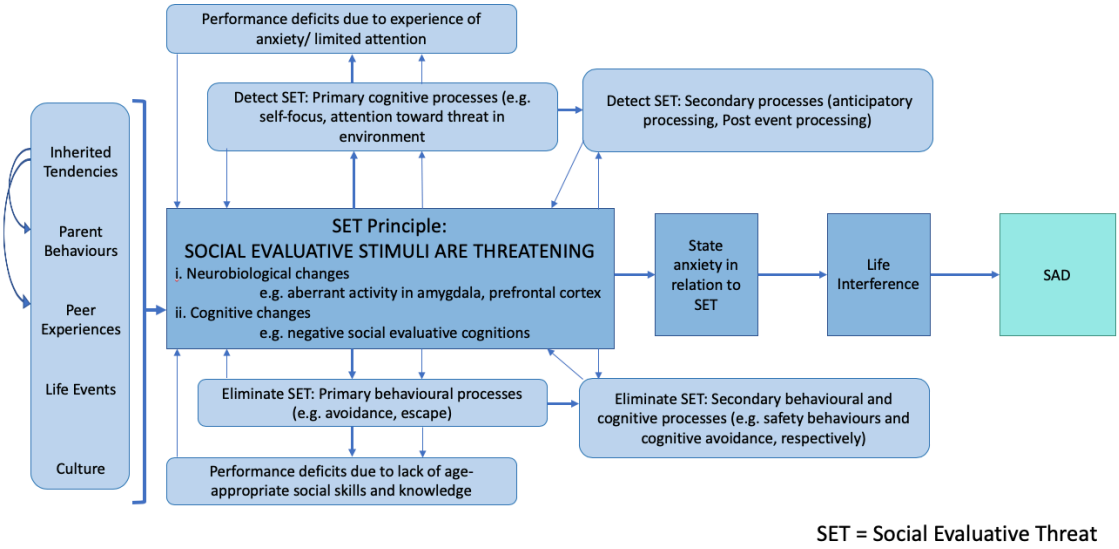


Figure 8.2. The integrated aetiological and maintenance (IAM) model of SAD (bold arrows indicate aetiological pathways; adopted from Wong & Rapee, 2016).

8.2.3 The classification and treatment of SAD – Categorical vs. dimensional and the RDoC initiative.

Increased psychophysiological fear or threat reactivity in SAD is well documented and serves to classify SAD as an anxiety disorder. The experience of social threat in SAD promotes the autonomic and somatic defence mechanisms in the body and this response provides a tangible measurement of fear arousal in the body. In SAD, this exaggerated fear arousal is specific to social engagement. This same fear arousal in the body in response to stress stimuli has been shown as blunted in other anxiety disorders, such as GAD, panic disorder, and clinically anxious individuals with comorbid depression (Lang & McTeague, 2009). Evidence suggests that the more diffuse and chronic the anxiety experienced by the individual (determined by chronicity, clinical judgement, subjective reports of negative affectivity), then the more compromised this fear arousal system may become (Lang & McTeague, 2009). This indicates that a blunting of the fear arousal response is systematically more pronounced across the anxiety spectrum, with the

severity of anxiety coinciding largely with the level of fear arousal. In light of this, it is suggested SAD is classified according to a 'transitional diagnosis', as those with the disorder do not display the extreme level of focused fear or threat reactivity you observe in a specific phobia, nor do they display the defensive impairment (blunting of the response) seen in GAD or panic disorder (Lang & McTeague, 2009). While a transition diagnosis of SAD suggests that threat reactivity pertains specifically to social engagement and is not incurred outside of social engagements, this thesis demonstrates that despite the threat reactivity in SAD being specific to social engagements the ongoing negative subjective impact of SAD is pervasive and significant across daily life and not isolated to the immediate social context. This opens the discussion for the best methods of diagnosis and classification of SAD, with classification of mental health disorders an ongoing debate in the literature. Current diagnostic systems rely heavily on categorical approached to diagnoses, and this next section will discuss categorical (the presence of absence of symptoms) vs. dimensional approaches (symptoms described along a severity continuum), and the Research Domain Criteria (RDoC) initiative from the National Institute for Mental Health (Insel et al., 2010).

Research has indicated that categorical assumptions of mental health disorders are problematic (Krueger & Eaton, 2015). For SAD specifically, sub-threshold manifestations of social anxiety have been associated with significant distress and functional impairment despite the absence of diagnostic criteria having been met, and there are important differences in severity of symptoms experienced within a diagnosis of SAD. Additionally, SAD is highly comorbid, and management of the disorder would benefit from being understood according to the unique presentation of the individual. Current systems of diagnosis and classification rely heavily on a categorical approach to classification (e.g. Diagnostic and Statistical Manual of

Mental Disorders (DSM) and the International Classification of Disease (ICD) systems; American Psychiatric Association, 2013; World Health Organisation, 1992, respectively). In the clinical domain, the highly variable aetiology and symptomology of SAD is classified under the label of SAD (e.g. symptom profile, severity, fluctuation, context). The foreword of the DSM-5 states the criteria for diagnosis are 'concise and explicit' – however, it is possible this kind of dichotomous 'in or out' classification limits our understanding and treatment of SAD. With the DSM-5 no longer including a significance criterion for SAD (e.g. mild, moderate, severe), and only a performance specifier, the classification of SAD does not clearly distinguish between severity of symptoms, nor their pervasiveness (i.e. one social situation or all social situations). The categorical classification also does not allow space for individuals who experience a degree of social anxiety across a number of different social situations and are at risk for development of SAD but have not yet reached clinically significant impairment. While not obviously meeting the clinical significance criterion, these individuals arguably still have significant symptoms and potential dysfunction in their social engagement, and importantly are still at risk for comorbidities. Individuals with SAD may also present with very different aetiological factors contributing to disorder onset and varying degrees of symptomology (e.g. severity). A categorical approach can gloss over the complexity of this disorder – both the overt symptoms and the underlying mechanisms of dysfunction. The difficulty of categorical classification of SAD, is that while symptoms of the disorder are often measured dimensionally, the categorical 'cut-off' for clinically significant distress or impairment used for diagnoses of SAD can be arbitrary. Contrastingly, a dimensional approach when assessing symptoms of social anxiety gives credence to the dimensional nature of the disorder and its symptomology. The dimensional approach is complex, and the complexity of this approach means it can be limited by the

challenge of articulating these dimensional profiles in a usable format for clinicians and researchers.

It is important in a highly variable disorder such as SAD, to not only understand the inter-variability between the disorder and the typically healthy experience, but also to examine the intra-variability that occurs within those that experience SAD. If there is a large proportion of intra-variability within the SAD diagnostic category, then understanding these differences may serve to inform why up to half of the treatment seeking SAD individuals do not respond to treatment. If the profile of SAD is vastly different across the diagnostic category, and social anxiety exists across a spectrum not a dichotomous category, then the treatment options for social anxiety must also cater to these differences in order to appropriately target the dimensional symptom profile. A one-size-fits-all overly simplistic categorical approach may miss nuances of social anxiety, forgoing the opportunity to understand the individual experience of social anxiety and target that experience directly. Mental health research continues to recognise many disorders share a number of core features, and effective targeted psychological intervention towards these core features, regardless of diagnoses, can be implemented (Hudson & Rapee, 2008). There are a number of shared core features for assessment and treatment that are relevant to all anxiety disorders, including anxiety triggers or cues (e.g. cognitive or situational cues), avoidance (e.g. situational and experiential avoidance), specific behaviours (e.g. safety behaviours or compulsions), physiological responses, and broader moderating factors such as, skill level, environmental and familial factors and medical concerns (Antony, 2002; Antony & Rowa, 2005). Future research should explore whether SAD is best treated according to the overt symptoms presenting in each individual along a dimensional approach, compared to the current categorical classification of SAD.

An emerging dimensional approach for psychiatric classification is the Research Domain Criteria (RDoC) initiative (Cuthbert & Kozak, 2013). The initiative suggests existing classification systems were established prior to advances in neuroscience, with the RDoC proposing a research framework that encourages a dimensional approach for the study of genetic, neural, and behavioural features of mental disorders. The RDoC provides cognitive and social processes, negative and positive valence systems and systems for arousal and regulation as the primary neurobehavioural mechanisms that serve the motivational and adaptive needs of the organism. The approach suggests a focus on the neurodevelopmental nature of mental disorders will serve to break free of categorical diagnostic constraints and capture associations between observable behaviours and symptoms, specifically the relationship between genetic and neural factors to behaviour (Cuthbert & Kozak, 2013). Importantly, the overarching goals of the RDoC initiative is to inform clinical trials, identify new treatment targets for improved intervention, define meaningful subgroups within a disorder for target treatments, and provide new avenues for research findings to translate to informed clinical decisions. The RDoC approach emphasises that classification systems like the DSM, while having contributed greatly to the reliability of mental health diagnoses and provided a framework for research and clinical domains, these systems were established prior to modern neuroscience and are now outdated (Insel et al., 2010). While it is a lengthy and slow process to develop any universal classification system, it is important for novel initiatives, such as the RDoC, to continue to generate new perspectives toward understanding and treating mental illness and shift the thinking around conceptualising mental health disorders to include modern advancements in research and new methods for the understanding the underlying mechanisms of mental health disorders.

8.2.4. Normalising the stress response in SAD. Our research demonstrates via reports of heightened negative affect, reduced positive affect and self-esteem, that the experience of acute social stress had significantly greater subjective impact on those with SAD than on the healthy control group. This supports current knowledge for the presence of a heightened negative interpretation bias in SAD, emphasising that individual's with SAD interpreted the social stressor more negatively than healthy controls, and reported greater overall subjective impact on a number of outcomes following the experience of social stress. What is interesting is that the greater subjective impact reported in SAD in response to acute social stress, is not reflected in greater physiological impact in regard to HPA axis activity, where the response between groups was comparative. Physiologically, other than evidence for altered cardio regulation in the form of reduced heart rate variability (HRV) in SAD when compared to controls (see Alvares et al., 2013; Gaebler et al., 2013; Pittig et al., 2013), the stress response in SAD is physiologically 'normal'. Understanding why subjective reports of acute stress in SAD are significantly more negative and the subjective impact of acute stress in SAD is far greater, despite physiologically the experience of acute stress being relatively comparative to healthy controls, has important clinical implications for the management of SAD.

One explanation for the subjective differences reported by those with SAD in response to stress may be maladaptive arousal appraisals of the typically normal physiological stress response. The typically normal human stress response results in increased feelings of arousal in the body due to the activation of the stress response systems following stress stimuli (see Figure 8.1 for the human stress response). With the known negativity bias in SAD, it may be that the interpretation of this adaptive stress response and the increased arousal in the system in response to stress is interpreted as a far greater stressor by those with SAD, compared to healthy controls.

One suggestion is that arousal reappraisal in SAD may contribute to improved stress responding and potentially change responses to negative evaluation in SAD (Jamieson et al., 2013). Cognitive reappraisal is used as an emotion regulation technique, speculated to assist with controlling stress responding and often utilised in CBT (Gross, 2002; Hofmann & Smits, 2008). The idea of arousal reappraisal, as suggested by Jamieson et al (2013), has the same common theme of intending to change cognitions in order to produce downstream benefits, with some important distinctions. Unlike emotion regulation techniques, the intention of arousal reappraisal is not to encourage SAD individuals to distance themselves from fearful stimuli or to reframe threat-provoking anxious situations (see Kross & Ayduk, 2011; Ochsner & Gross, 2008). Nor does arousal reappraisal encourage relaxation techniques during social evaluative threat situations (such as Goldin & Gross, 2010). Instead, the aim of arousal reappraisal is to normalise the 'normal' stress response. Individuals are encouraged to alter their perceptions of stress arousal in order to improve responses to stress by maintaining sympathetic activation (Jamieson et al., 2013), with sympathetic activation said to facilitate effective coping and performance across acute stress. Individuals with SAD are encouraged to normalise the physiological response to social stress, and change their perception of the typically normal stress response (e.g. changes in heart rate, breath, arousal in the system) through arousal reappraisal. Further, they are encouraged to enter social engagements with the expectation of a proportional degree of concern around others judgement or scrutiny when exposed to socially evaluative threat and uncontrollability (Dickerson & Kemeny, 2004). The arousal reappraisal and expectation of a degree of social anxiety during social engagements may serve to change perceptions around the stress response being overwhelmingly negative in SAD. In normalising the experience of social stress through arousal reappraisal, the interpretation of subjective stress may not continue

with the same degree of distress typically reported. Traditional CBT approaches could be helpful to assist with arousal reappraisal. With the most common and effective treatment for SAD currently being CBT techniques, with a central focus on cognitive restructuring and exposure, encouraging those with the disorder to reappraise how they view sympathetic arousal in their system during stress may be a useful therapeutic addition. In conjunction with typical cognitive restructuring (e.g. flexibility in thinking or challenging unhelpful interpretations) and emotion regulation (e.g. cognitive reappraisal), encouraging arousal reappraisal during exposure work may lead to improved stress responding in the moment. Emerging therapeutic approaches, such as acceptance and mindfulness approaches (Hofmann, Sawyer, Witt, & Oh, 2010; Niles et al., 2014), could also be useful in this kind of approach toward normalising the experience of stress in SAD.

8.3 The Strength of This Thesis – Daily Life Research in Social Anxiety Disorder

8.3.1 Beyond the lab. Clinical psychology has established time-dependent fluctuations in affect and self-esteem as distinct features of a number of clinical conditions (e.g. depression and PTSD; Kashdan et al., 2006). Despite the time-contingent and context-dependent nature of SAD symptomology, this thesis has highlighted that the existing body of research into the subjective experience of the disorder has predominantly focused on trait or mean levels of psychological processes and is heavily based on laboratory assessment (see Chapter 2). Much is still to be discovered about the temporal and dynamic patterns of SAD symptomology in daily life and how this disorder unfolds in daily life (Farmer & Kashdan, 2014).

From the variation in the subjective reports observed across the phases in this thesis (baseline, and the anticipation of and recovery from social stress), we highlight the benefit of real-time assessment in SAD, particularly of social engagements, to

capture the fluctuating and time-contingent mechanisms that contribute to distress and the maintenance of the disorder. The capture of these maladaptive processes in close proximity to the feared stimuli (e.g. social engagement) may potentially identify critical targets for therapeutic intervention in future treatment approaches that are otherwise lost in static single assessment modalities (Geyer et al., 2018). EMA is increasingly being used in studies as a way to track people's thoughts, behaviours, emotions, and the context of these processes within their natural environment. By utilising daily life research methodology, this research was able to observe the broader profile of the adverse subjective experience of acute social stress. We provide new insights into how the immediate subjective response to acute social stress in SAD also has a 'spill over' effect that permeates across daily life, outside of the immediate social stress context. While social anxiety is specific to the social context, the heightened negative affect, reduced positive affect, lower self-esteem and greater threat awareness and fear of negative evaluation were pervasive across time and consistently so, when compared to healthy controls. We also demonstrate reactivity in some subjective measures, both inside and outside of the lab. Those with SAD were more reactive in their reports of anxiety and happiness during the completion of the TSST (Study 1), and this lab-based reactivity observed in anxiety and happiness was also reported in daily life during the anticipation of and recovery from the acute social stressor.

Further, heightened reactivity in appearance satisfaction (during anticipation and recovery) and anger (during recovery only) in SAD was also demonstrated compared to controls. This thesis therefore demonstrates the anticipation of and recovery from a social engagement led to changes in some subjective measures in SAD, but not all (i.e. sad, embarrassed, self-efficacy, fear of negative evaluation, threat awareness and environment security) with just the four measures (anxiety,

anger, happiness and appearance satisfaction) differing in the anticipation or recovery phase in SAD, when compared to baseline and healthy controls. In examining the subjective experience of those with SAD around an acute social stressor, we demonstrate that while those with SAD were significantly worse across the board in their subjective reports, this more negative, less positive experience in SAD was consistent across time for most measures.

Evidence from this thesis, specifically the higher anxiety and anger and lower happiness and appearance satisfaction during the *recovery* phase following acute social stress, may support the theory of heightened negative post-event processing in SAD (Farmer & Kashdan, 2015). In order to explore mechanisms highly relevant to SAD, such as post-event processing, research needs an assessment methodology that can capture these mechanisms as they unfold outside of the immediate context of the social engagement, with EMA providing an accessible way for research to do so. Through the use of repeated assessment across time following a social stressor, results from this research supports that individuals with SAD possibly spend a greater amount of time recalling and analysing their own behaviour than that of others during the social stress task long (i.e. days) after the engagement itself has ceased. Negative post-event processing often focuses on flaws or errors that might contribute to the fear negative evaluation or scrutiny (Brozovich & Heimberg, 2008). Our research offers insight in post-event processing in SAD and suggests the possibility that an increase in negative self-focused attention during and following an acute social stressor may contribute to increases in negative affect. It is possible those with SAD engaged with greater negative post-event processing compared to the healthy control group and this consequently led to the greater anger and anxiety, and less happiness and appearance satisfaction following social engagement that was observed in SAD. This is in line with existing literature on post-event processing in SAD, where significant post-event

processing has been demonstrated in the disorder and the severity of post-event processing positively associated with level of anxiety (e.g. Kocovski & Rector, 2008). It is widely understood that cognitive processes, such as negative post-event processing and self-focused attention, can occur for extended lengths of time (up to days) following the occurrence of a social event, such that those with SAD are expected to experience the effects of social stress for an extended duration, as observed in the recovery phase (Farmer & Kashdan, 2015). The understanding of post-event processing possibly continuing for days post-conclusion of an event highlights the potential benefit of using daily life assessment to examine the broader profile and real-world fluctuation in these processes across an extended time frame. Interestingly, when examining the IAM model of SAD (Wong & Rapee, 2016) and the need for future research to test the model, EMA methods would be particularly useful for future research to examining the social-evaluative threat principle, changes in cognitions, the use of primary behavioural processes (e.g. avoidance and escape behaviours in daily life), anticipatory processing and post-event processing, cognitive processes (e.g. use of safety behaviours and cognitive avoidance), fluctuating level of state anxiety and life interference across the daily life of SAD.

8.3.2 Contribution to treatment advances. Regarding the use of daily life assessment in this thesis and potential advancement in treatment, this research demonstrated that the higher levels of negative affect, lower level of positive affect, diminished self-esteem and higher levels of threat awareness in SAD are both pervasive across time, but further, that some measures of the subjective experience in SAD vary across time as a consequence of social stress (i.e. anxiety, anger, happiness and appearance satisfaction). Being able to identify what may contribute to these experiences in real-time as they occur, and the context in which they occur, allows for researchers to think how these specific factors may be targeted for treatment in the

moment as they occur. This is in contrast to current methods that wait for individuals to seek out treatment, something that typically occurs well-after the onset of SAD and likely after the maintaining mechanisms of the disorder have extensively occurred (i.e. negative post-event processing). This novel form of treatment, known as 'just-in-time' intervention, aims to accommodate the immediate needs of an individual in the moment, with advancements in mobile technology offering new and exciting opportunities to do so (Riley et al., 2011). While just-in-time interventions are still in infancy, the widespread everyday use of smartphones and advancements in software development and algorithms make this form of intervention more feasible by the day (Nahum-Shani, Hekler, & Spruijt-Metz, 2015). Mobile health interventions have provided timely support for a wide range of health behaviours, including mental health (e.g. schizophrenia; Ben-Zeev et al., 2014). The use of time-contingent methodologies, such as EMA, to inform time-contingent treatment presents exciting possibilities for early intervention and deeply personalised treatments that identify the individual's maladaptive cognitions and behaviours as they happen and seeks to rectify or assist in reducing these before they develop into ingrained constructs in the individual that prove resistant to change (Geyer et al., 2018). Future SAD research should investigate how this rich EMA data is transferrable to just-in-time intervention strategies in real life.

8.4. General Limitations and Future Directions

While the specific limitations associated with each empirical study have previously been discussed in the relevant chapters (see Chapters 6 and 7), it is important for the overall contributions of this thesis to be considered in light of several constraining factors, including: potential attribution errors, refining the scope of this thesis, using technological-based assessment, limited physiological assessment

for discussion of the different stress response systems (i.e. autonomic stress response), other individual factors, and reporting a sample free of comorbidity.

8.4.1 Scope of this thesis. We firstly acknowledge the scope of this thesis as a potential limitation. For example, the behavioural mechanisms of SAD are important for future research to examine, with the understanding of social behaviours (e.g. who time is spent with, frequency of social engagement, environment time is spent in) in SAD of great value for understanding impact of the disorder in daily life and possible implications of behavioural change (e.g. use of safety behaviours like avoidance). While this project did obtain information about where participants spent their time (e.g. the environment) and with who (e.g. family) across daily life, examination of these behavioural and environmental factors was beyond the scope of the specific research questions of the empirical studies included in this thesis. We make special note that the possibilities of EMA data are vast, particularly as this project sampled variables outside of those presented in Study 2 during the mobile app monitoring. The reach of this project was expansive, and a clear decision of the scope this thesis had to be refined. While there remain numerous possibilities within this data set to explore in SAD, there had to be reasonable constraints to the scope of this research and the specific research question, which will likely be addressed in later publications.

8.4.2 Potential for an attribution error and the value of a larger sample size. One limitation of the results of this thesis is that we have suggested the observed changes in daily life are as a result of engagement in an acute social stressor. We note that there are many other factors in the daily lives of participants with SAD that could be predictors of these changes in subjective report that were observed. For example, participants with SAD may have spent their time before and after the social stressor in the company of familiar versus unfamiliar people, alone or in public, at work or on a day off, all of which could influence their affective experience. Thus, the experiences

we reported here may not be exclusively in response to the social stressor, i.e., the TSST. Future research could include other factors specific to the social context (i.e. with whom time was spent, where time was spent) to further clarify which features of daily life may contribute to fluctuations in SAD. Future research could examine these factors specifically to understand how the company kept and the location where time is spent is associated with the experience of SAD and management of social stress.

Regarding factors that were not explored in this thesis, we highlight at times the most promising gain of EMA data can also be its great challenge. EMA data is insurmountable in its ability to provide rich and descriptive information about real-world experiences, however, the sheer amount of data and the potential different analyses and avenues of investigation are enormous. It is a challenge in itself when presented with so many possible outcomes, and therefore clearly refined research questions, ideally preregistered (e.g., on OSF Preprints; https://osf.io), would be of great benefit in this type of research. We acknowledge many potential avenues of exploration into SAD exist within this data set. Our choice was to examine the acute stress response in SAD in daily life as our contribution to the literature, and thus contained our research to this specific domain.

Regarding power, we address a limitation in the sample size of this thesis. While 40 clinical SAD and 40 healthy control participants may be an adequate sample size and a substantial recruitment and testing effort for some components of this thesis, EMA data sets benefits from significantly larger sample sizes. Simply put, the larger the sample in EMA, the more factors can be included in analyses. There was a fine balance between the practical management of this methodology (e.g. lab-based assessment requirements, 8-day EMA protocol, participant burden, at-home saliva sampling) and achieving substantial enough numbers for the daily life EMA data set.

8.4.3 Physiological assessment. While the literature is not explicitly clear on the relationship between SAD and the physiological acute stress response, our research should be considered in light of the limited physiological sampling scope. Salivary cortisol was the only physiological measure included within this thesis. While buccal cells (for genetic sampling and DNA analyses), heart rate variability and heart rate during the TSST and endogenous hormones were collected for this research project, this data was not included in these analyses as it was beyond the feasible boundaries of this thesis. Much of the stress research in SAD included different physiological measures or multiple measures of interest (e.g. sAA as a measure of SAM axis activity, other autonomic assessment, fMRI, blushing, galvanic skin response). While our current data provides insights in HPA axis responding and discusses this while drawing on existing evidence for differences in autonomic stress responding in SAD (e.g. heart rate variability), this research cannot explicitly talk to a number of measures of physiological stress in SAD and cannot resolve the existing inconsistencies in stress literature (outside of the HPA axis). Lab-based psychophysiological assessment has provided enormous insights into the aetiology and maintenance of mental health disorders, however, can demonstrate lower ecological validity and reduced real-world application. Much like the advances in subjective EMA data collection, psychophysiological assessment is also moving toward improve ambulatory recording of psychophysiology, paired with EMA techniques (Raugh, Chapman, Bartolomeo, Gonzalez, & Strauss, 2019). Future research may seek to clarify the potential moderators of the physiological stress response that we were unable to include. Further, with rapid advancement in EMA techniques and the sampling of psychophysiological in daily life more accessible and achievable in the current day, future research may seek to sample a broader range of physiological measures (e.g. other endocrine measures or analytes of interest,

electrocardiography, blood pressure, electrodermal activity) in response to acute stress in daily life in SAD. For a recent review of the psychophysiological applications for EMA in clinical populations see Raugh, Chapman, Bartolomeo, Gonzalez, and Strauss (2019).

8.4.4 Comorbidities. A potential limitation of this research is also the presence, or reported lack there-of, of comorbidities in the SAD sample. The current study used a sample of SAD individuals who, at the time of participation, reported no current comorbidities or secondary diagnoses during screening. While during the screening process all efforts were made to control for comorbidities in the thesis sample, observation of the mean group scores on additional clinical outcome measures that were assessed post screening, such as the DASS-21, demonstrate that the clinical SAD group surpassed the 'extremely severe' severity cut-off scores on all three scales of depression, anxiety and stress. The presence of comorbidity is a potentially noteworthy moderator of our results, particularly comorbid depression (Jamieson et al., 2013). In this thesis, we did not directly examine symptoms of depression in relation to the response's participants exhibited in our two empirical studies. Existing evidence suggests that the physiological reactions of individuals with social anxiety may be dependent upon comorbid depression (Yoon & Joormann, 2012). We also note that the use of the MINI (Sheehan et al., 1998) as a screening device may not have the sensitivity of other screening tools, such as the SCID (Spitzer et al., 1992). However, the MINI was the most practical and less time constraining screening tool for use in this thesis. Future research into the acute social stress response in SAD may consider examining comorbidities and the potential influence these may have on the reactions exhibited by SAD individuals with and without comorbidity.

In the absence of comorbidity, if we are to take our screening of participants and their responses at face value, then we have a sample of SAD individual without comorbidity. Physiologically, while this theoretically contributes to the understanding of the underlying mechanisms of SAD in isolation, without the influence of potentially moderating factors of other disorders (e.g. dysregulation of the HPA axis observed in GAD, MDD, PTSD, see Adam et al., 2014; Ngampramuan, Tungtong, Mukda, Jariyavilas, & Sakulisariyaporn, 2018; Pan, Wang, Wu, Wen, & Liu, 2018), it may limit the generalisability of the results. It is well known that SAD is a highly comorbid disorder (American Psychiatric Association, 2013), as such when considering the implications of this research and how it transfers to real-world clinical applications, it should be considered whether the absence of salivary cortisol differences observed between SAD and healthy adults in this study apply differently to those who experience SAD with or without comorbidities. Subjectively, consideration for how these results of the subjective experience of acute social stress apply to SAD individuals with the presence of comorbidities (e.g. MDD) should also be explored, and future research may benefit from directly including additional clinical measures in analyses (e.g. depression severity scales) in order to identify potential associations between SAD, other mental health symptoms (e.g. depressive symptoms) and the stress response. SAD is a highly comorbid disorder and evidence suggests those with severe social anxiety, when compared to those with depression, appear to be differentially influenced by negative and positive affect and the intensity of the affect that is encountered during social engagements (Geyer et al., 2018). This suggests that the effects found may differ according to the presence and severity of depressive symptoms in SAD, as such, these findings should be replicated in SAD individuals with and without comorbidities, in order to understand how the presence or absence of comorbidities may influence the experience of social stress in SAD.

8.4.5 Technological and logistical difficulties. As with any use of technology and complex research project, there has to be consideration of the limitations that may arise. The complex nature of this methodology and the use of a custom smartphone phone application on participants own smartphone came with a number of challenges and required additional time demands and training of participants. Participants needed to be well-versed in the procedures and tasks they were required to follow and have a thorough introduction to and training for the use of the testing equipment involved. It was also the responsibility of the lead researcher to monitor and address participant compliance and motivation across each individual's participation and emphasise to participants the importance of the study their contribution to the research (Shiffman et al., 2008). Missed surveys, delays in commencement of surveys, sync delays, and problems with notifications on the android (vs. iOS) interface were all present in this study that inevitably resulted in a reduced number of surveys completed by some participants. These examples speak to the importance of large sample sizes in EMA data due to the potential exclusion of a number of problematic responses or missed surveys during the data cleaning process.

Another potential limitation of our EMA research is measurement reactivity, and the potential for the subjective measures of interest to change as a result of being studied (Wheeler & Reis, 1991). Increased attention toward anxiety or worry may potentially trigger increased cognition or rumination around these anxieties which may lead to increases in these states (e.g. Mathews & MacLeod, 1994). Conner and Reid (2012) also report reactivity effects on the reporting of mood and subjective experience when using mobile technology. It is also pertinent that future EMA in SAD research continue to explore whether EMA is in fact measuring a phenomenon as is, or if the phenomenon is transformed by measurement itself and further, whether reactivity differs across the different groups and variables measured through EMA

(Scollon et al., 2009). We note a limitation of our survey is that the integrated questions were not specific to the acute social stressor. We sampled daily life affect, self-esteem, threat awareness, and environmental security, but did not explicitly ask these in reference to the acute social stressor. This serves as a reminder for future research to ensure their sampling is explicitly asking questions that directly relate to the research questions. We also attempted to control for reactivity effects and effects of other potential influences (e.g. weekend/week-day) by using the *cleansema* program (Murphy, 2017), which cleaned the raw data according to standard guidelines for reaction time, missing data, implementing weekend and weekday classification, checking time stamps and other data checks.

8.4.6 Diagnostic cut-offs vs. dimensional assessment. As we note in section 8.4.4, a potential limitation of this study is the report of a 'pure' sample of SAD individuals, in that no comorbidities were reported by SAD participants, nor were diagnostic criteria met for other disorders during screening. When observing the clinical characterisation of the SAD group however, we note scores on other clinical outcomes such as the depression, anxiety and stress scale (Lovibond & Lovibond, 1995) saw the group mean of the SAD participants meeting the severe-to-extreme classification cut-off. While this study focused primarily on between-group differences between SAD compared to healthy controls, there is evidence to suggest there is also great variability within the experience of SAD also. It is important for future research to determine if diagnostic differences exist within the SAD category, related to the varying profiles of subjective, psychological, cognitive, neurological, behavioural and comorbid factors that exist within the one diagnostic category. Examining the intra-variability that may exist within our SAD group was beyond the scope of this thesis, however, future research may look at differences in the physiological and subjective experience of acute stress within the SAD group (e.g.,

different severity levels, and different social triggers such as performance vs. social environments), in addition to the comparison between SAD to healthy controls we completed. Future research should examine the possibilities of categorical vs. dimensional classification of SAD and what information may be missed in the use of these two approaches dichotomously. Future research may potentially consider advancements that employ tandem categorical and dimensional assessment, with rigorous evaluation for what the appropriate cut-off points for disorder classification and treatment response may be (e.g. Bandelow, Baldwin, Dolberg, Andersen, & Stein, 2006).

8.5 Conclusion

Daily life assessment via EMA is not the holy grail of methodologies and does not resolve all of the pitfalls and challenges in psychological research. As with all methodologies, EMA is filled with as much promise as it is hindered by challenges. What it does offer however, is a way to capitalise on technological advances and provide unprecedented access to the experience of SAD in daily life. With careful management of the challenges of EMA and tailoring of the method to the individual research project, EMA can contribute enormously to our understanding of SAD. This thesis provides evidence as a first step in the direction of integrating tech into SAD research. A key facet to understanding mental health is an in depth understanding of the naturally occurring stability and variability in the experience of the disorder (Gloster et al., 2017; Kashdan & McKnight, 2011; Thompson et al., 2012). EMA provides an opportunity to cultivate this kind of insight and can inform decisions around diagnostics (i.e. symptom frequency and length) and treatment outcomes. The potential access and insights EMA may provide far outweigh the pitfalls of the methodology (i.e. time commitment, resource demands, expenses and participant

burden). This thesis aimed to utilise EMA to contribute to the understanding of the processes and mechanisms of dysfunction underlying the acute social stress response in SAD and add to the understanding of symptom variability and fluctuation in the disorder.

This thesis contributes several aspects to the understanding of SAD, including (i) confirming a 'normal' HPA response to social stress despite heightened subjective stress evident in both the lab and daily life, (ii) greater reactivity to social stress across daily life in not only measures of negative affect (i.e. *anxiety* and *anger*) but also positive affect (i.e. *happiness*), (iii) reactivity only in *appearance* based self-esteem in response to social stress, with *self-efficacy* and *fear of negative evaluation* measures of state self-esteem consistently low but stable, (iv) individuals with SAD were significantly and consistently worse compared to healthy controls across the board in all subjective reports of affect, self-esteem, threat awareness and environmental security throughout the entire week of participation. While some measures varied across the phases in the anticipation of and recovery from engaging with an acute social stressor (i.e. anger, anxiety, happiness, appearance satisfaction), this research demonstrates the experience of SAD is pervasive across time and the repercussion of the disorder are not isolated to the social context but observed broadly throughout daily life. With a richer understanding of SAD in daily life, clinicians may be afforded the ability to tailor treatment specifically to the individuals and target symptoms in the moment, as opposed to long after negative evaluations of the self, rumination of social engagements and maladaptive behaviours have occurred – utilising novel 'just in time' intervention approaches via daily life methodologies. This thesis highlights the importance of understanding the nuances of how those with SAD experience social stress subjectively, i.e. their subjective reality, as a way to individualise treatment to the individual and the relevant mechanisms contributing to their personal distress.

In beginning this research project, it was revealed that a detailed understanding of the naturally occurring picture of SAD in daily life was lacking, particularly in response to acute social stress. This thesis intended to highlight the benefit of utilising a multimodal approach for studying SAD, particularly due to the symptomology being context dependent (i.e. fear or anxiety experienced is socially based) and fluctuating across time (i.e. in relation to proximity to a social encounter). Capturing the experience of social engagement in daily life outside of their immediate context presents a unique way to understand the ongoing impact of these social stressors and how those with SAD continue to experience acute stress after the immediate stress has ended. In investigating the broader impact of acute psychosocial stress outside of the lab environment, we produced complementary evidence about the nature of the acute stress response in SAD and how individuals with SAD respond to social stress. We highlight the dysfunction in the disorder is primarily in the subjective experience of those with SAD, with the physiological (salivary cortisol) stress response comparative between groups. The multi-modal approach of this thesis capitalised on the strength of both lab-based and daily life methodologies and delivered evidence for the nature of SAD and the acute stress response in two complimentary lines of inquiry. Through this approach we add to a richer understanding of the underlying mechanisms at play in SAD that contribute to the development, maintenance and chronic nature of the disorder. Mental health is a real-world experience, and there is unrivalled potential for daily life methodologies to explore the reality of mental health disorders as they unfold in every day to day living.

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Appendices

Appendix A – Study Materials

Appendix A – 1. Advertising flyers

a) Recruitment Flyer for Healthy Controls.

Are you Over 18?

We are seeking individuals to participate in a study investigating the relationship between hormones, brain activity and social behaviours.
You will be reimbursed for your time and effort!

ACU
AUSTRALIAN CATHOLIC UNIVERSITY

Cognition & Emotion Research Centre

YOU CAN HELP CONTRIBUTE TO THE RESEARCH OF SOCIAL ANXIETY DISORDER BY PARTICIPATING IN A RESEARCH PROJECT!

Using cutting-edge mobile technology to monitor feelings, behaviour, and physiology in the 'real world' alongside laboratory exploration of social behaviour and functioning, we aim to understand several key aspects of emotional functioning in daily life. The study involves 3 parts:

- **Lab-based sessions:** Two 1-1½ hour sessions will be conducted in the laboratory at ACU (Melbourne Campus, Fitzroy). You will complete some background and well-being questionnaires, and a social task.
- **Mobile App monitoring:** Using an app on your smartphone (android/iPhone) we will monitor your feelings and behaviour via a series of prompts that involve answering a brief questionnaire multiple times per day across 7 days (~15 mins per day).
- **Saliva collection:** During the mobile app monitoring, you will also complete at home saliva sampling for 3 of the 7 days.

You can participate, IF:

- No current *OR* SUSPECTED diagnosis of mental illness
- Are right-handed
- Between the ages of 18 - 55 years
- A non-smoker & no drug/alcohol abuse
- Medication free (ideally)
- Have no metal objects present in body (or which can't be removed)
- Own a smartphone (android or ios)

Interested?

Contact Caitlin Grace via EMAIL at:
caitlin.grace3@myacu.edu.au

Participation is voluntary and you can withdraw at any time.

You will be reimbursed for your time and effort!

***Ethics approval: HREC* 2016-217H**

b) Recruitment Flyer for SAD.

Appendix A – 2. Pre-testing phone screening and demographics questionnaire

Script: "Thank you for your interest in participating in this exciting study. The first step is a phone screening and demographics questionnaire in order to determine your eligibility for this study. It will take approximately 5 minutes. Is this a suitable time for us to complete this or is there a more convenient time you would like us to call you back?"

Date:_____ **Age:**_____ **D.O.B.:**_____

Screened by: _____ ID to Identify participant by: _____

Contact Details of Potential Participant kept separately to screening details

Instructions: *Now I will need to go through several questions with you. These questions are designed to help us understand any medical problems that you may have and to determine your suitability for the study. All information given will be treated in the strictest confidence.*

Participant Group: Healthy Control ☐ Social Anxiety Disorder ☐

Are you male or female? Male ☐ Female ☐

Are you Right-handed? ☐ Yes ☐ No → **X**

Are you Non-smoker? ☐ Yes ☐ No → **X** → # of cigarettes / day

Do have any <u>metal objects</u> in your body which can't be removed or not MRI safe? ☐ Yes ☐ No

A Cardiac pacemaker ☐ Yes ☐ No

A Prosthetic heart value ☐ Yes ☐ No

A surgical clip, bone or joint replacement, or any metallic implant ☐ Yes ☐ No

Have you at any time held a job in a metal-working industry or one in which you may have been exposed to metallic dust or splinters ☐ Yes ☐ No

Have you suffered a shrapnel wound ☐ Yes ☐ No

Any metallic chips or splinters in the eye ☐ Yes ☐ No

Are pregnant, or think you might be (females only) ☐ Yes ☐ No

Weigh more than 300 pounds (136 kgs) ☐ Yes ☐ No

Suffer from claustrophobia ☐ Yes ☐ No

Any body piercings ☐ Yes ☐ No

Make note of objects which will need to be removed: _____

Do you take any hormonal <u>contraceptives</u>? ☐ Yes ☐ No

Do you take any <u>medications</u> (prescription or over-the counter)? ☐ Yes ☐ No

If yes, please fill in the details in the table below:

Name of medication	Dose	Number of times taken each day	Date of commencement

Criteria: Psychotropic medication free for min 2 wks (8wks for fluoxetine, 4 wks for MOIs)

Do you have history of <u>psychiatric illness</u> or current diagnosis *<u>PERSONALLY</u>*? ☐ Yes ☐ No

Anxiety or depression (in last 6 months)? ☐ Yes ☐ No

Any other psychological problem? ☐ Yes ☐ No Details:_____

Do you have history of psychiatric illness or current diagnosis in your *<u>FAMILY</u>*? ☐ Yes ☐ No

Bipolar ☐Yes ☐ No

Schizophrenia ☐ Yes ☐ No

Obsessive Compulsive Disorder (OCD) ☐ Yes ☐ No

Post-Traumatic Stress Disorder (PSTD) ☐ Yes ☐ No

Anxiety or depression? ☐ Yes ☐ No

Any other psychological problem? ☐ Yes ☐ No

Details: (type and who) _____

Do you have any of the following <u>medical problems</u>? *If yes, please give details.*

Heart problems? ☐ Yes ☐ No
High or low blood pressure? ☐ Yes ☐No
Respiratory problems? ☐ Yes ☐ No
Stomach or intestinal problems? ☐ Yes ☐ No
Liver problems? ☐ Yes ☐ No
Kidney or urinary problems? ☐ Yes ☐ No
Diabetes? ☐ Yes ☐ No
Anaemia or blood disorders? ☐ Yes ☐ No
Epilepsy or fitting? ☐ Yes ☐ No
Eyesight problems or colour blindness? ☐ Yes ☐ No
Cancer? ☐ Yes ☐ No
Skin disorders? ☐ Yes ☐ No

Do you currently suffer from any cold or flu symptoms? ☐ Yes ☐ No
If yes, when did it start: _____and are you taking medications_____
Do you or have you used any illegal drugs/substances before? ☐ Yes ☐ No
If yes, what type(s): _____
How often: _____
When was the last time you've used these? _____
Have you used anything in the last 6 months? ☐ Yes ☐ No → **X**
Do you drink alcohol? ☐ Yes ☐ No
If yes, # of glass = _____ / day / week. Main type: _____
When was the last time you had a drink: _____ Amount: _____
Do you follow any special diet? ☐ Yes ☐ No
If yes, what type? _____
Have you ever had any operations? ☐ Yes ☐ No
If yes, please give details: _____
When did you last consult a doctor? And for what reason?
Date of last doctor's appointment: _____ Reason: _____
Have you ever suffered a head injury and/or loss of consciousness? ☐ Yes ☐ No
If yes, did you have to go to hospital? ☐ Yes ☐ No
Were you unconscious for longer than 5 minutes? ☐ Yes ☐ No

Other:
Do you drink coffee? ☐ Yes ☐ No → # of cups /day = _____
Do you use glasses? ☐ Yes ☐ No → Contact lenses? ☐ Yes ☐ No
Do you use a hearing aid? ☐ Yes ☐ No
Do you use any other type of prosthesis? ☐ Yes ☐ No

Education:
a) At what age did you begin your education (e.g., Primary school)? _____
b) At what age did you finish your education? _____
c) What is the highest education standard you have completed? (e.g. primary, secondary, tertiary course, trade course, apprenticeship)_____

Employment
a) What is your employment status?
☐ Employed full time ☐ Employed part time
☐ Unemployed (but seeking work) ☐ Home duties
☐ Student ☐ Retired ☐ Disability pension
b) What is the highest level of employment you have achieved?
☐ Employed full time ☐ Employed part time
☐ Casual employment ☐ Never employed full time or part
c) What was the nature of the previous employment?

Appendix A – 3. Participant information letter

PARTICIPANT INFORMATION LETTER

PROJECT TITLE: Daily Emotional Functioning in Social Anxiety Disorder
PRINCIPAL INVESTIGATORS: Dr Izelle Labuschagne; Dr Gill Terrett; Dr Peter Koval; A/Prof Leah Brennan; Prof Peter Rendell; Dr Darren Hocking.
STUDENT RESEARCHER: Caitlin Grace
STUDENT'S DEGREE: Master of Psychology (Clinical)/PhD combined degree

Dear Participant,

You are invited to participate in the research study described below.

What is the study about?
This research study examines how emotional and anxious experiences in daily life, are related to basic thinking and biological processes in people with Social Anxiety Disorder (SAD). We will recruit individuals from two different groups: i) those with SAD, and ii) typically healthy controls. We hope to discover how the daily emotional lives of people with SAD, are shaped by underlying differences in biological (brain and hormones) and thinking processes (emotional and fearful experiences).

Oxytocin and *vasopressin* are two hormones that are closely related and produced in the same area in the brain. Oxytocin is best known for its role in pregnancy, and it is commonly administered to pregnant women to induce labour. Our research however is not interested in the pregnancy effects of these hormones, but instead how these hormones play a role in our everyday behaviours. Recent evidence has suggested that oxytocin and vasopressin play a key role in our social and emotional behaviours, including anxiety and fearful experiences, which are behaviours closely associated with SAD.

This research is novel and will provide answers to questions such as whether hormonal levels implicated in social and emotional behaviours are different between SAD compared to a typical group of controls, and whether these hormonal levels relate to variations in brain responses in SAD compared to typical controls. If you decide to take part, your involvement will give you an opportunity to use the latest cutting-edge tools for monitoring emotions in daily life (e.g., mobile phone app) and gain first-hand experience of scientific research (e.g., brain scan). Your time and efforts will help to promote scientific understanding of SAD in daily life, which will assist in developing new treatments for people with SAD.

Who is undertaking the study?
This study is being conducted by **Dr Izelle Labuschagne, Dr Peter Koval, A/Prof Leah Brennan, Prof Peter Rendell, Ms Caitlin Grace and Ms Laura Payne** and, from the Australian Catholic University and **Dr Darren Hocking** from La Trobe University. Dr Labuschagne is a neuroscientist with a strong interest in anxiety and emotion related mental health problems including understanding how these behaviours manifest in the brain. She also focuses her research on oxytocin and vasopressin. Dr Koval has extensive expertise with assessing emotional behaviours in daily life using mobile phone apps. A/Prof Leah Brennan is an experienced clinical psychologist with expertise in behavioural treatment programs in various clinical populations. Prof Peter Rendell is psychologist and director of the research group at ACU (Cognition and Emotion Research Centre). Ms Caitlin Grace is our enthusiastic

student researcher who has an Honours degree in Psychology and experience in conducting research using mobile phone apps with young adults. This study will form the basis of Caitlin's Doctorate degree and Laura's Honours degree in Psychology at ACU. Dr Hocking is a researcher that works on anxiety and neurodevelopmental disorders

What will I be asked to do?
You will first be required to complete a screening session to determine your suitability for the study. If you are suitable, you will be entering the study consisting of three phases. Following completion of Phase 1 you will be provided with the opportunity to sign on and continue into Phases 2 and 3. The three phases are described below.

PHASE 1 – *Introductory lab session, 7-day ambulatory assessment using a mobile phone app, and a social experiment.*

- During an initial *introductory lab session* (2 hours), you'll be asked to complete short questionnaires and an interview about your background and well-being, including about any psychological or medical problems. Following the questionnaires and interview, you will be provided with details about the ambulatory assessment component involving the mobile phone app. You will be asked to download a free smartphone app onto your own smartphone and you will receive instructions for using the app. You will have a chance to practice answering the smartphone surveys and can ask for further clarification about the smartphone survey from the researcher if required. You will also receive saliva sample collection kits. You will be instructed on how to collect your own personal saliva samples on 3 days during the 7-day ambulatory assessment. The lab session will take place at **the School of Psychology on the Melbourne campus of ACU (located at 11-14 Young Street, Fitzroy)** at a mutually convenient time.

- The *7-day ambulatory assessment* will involve doing the normal things you do every day, but you will be asked to answer survey questions using a smartphone app. The phone app will prompt you to complete a short survey that will ask about your current feelings, environment, any negative or positive events, and what strategies you've used to manage or deal with your emotions. This survey will be completed 10 times per day for 7 days. The first time you complete the survey it will take approximately 2 minutes, however after you've completed it a few times, you will become quicker at responding and each survey will only take about 1 minute to complete.

- The *social experiment* will involve another lab session (120 minutes). This will occur on Day 4 of your 7-day ambulatory assessment. On Day 4, you will be asked to return to the lab at ACU, and you will complete a well-established social experiment designed to elicit strong emotional responses, similar to what most experience in their lives, such as when going on a first date. During the experiment, we will record your anxiety and arousal levels via a self-report questionnaire and saliva samples (e.g., cortisol) as well as your heartrate. Most of the time will be spent with the main researcher collecting the data. However, there will also be a 10-minute active social part during which you will engage with other members. This will occur in a controlled and supported environment. At the end of this session, you will leave the lab and continue to complete your mobile phone surveys for the remaining 3 days of the 7-day ambulatory assessment. You will then be reimbursed for your time and effort accordingly.

At the completion of Phase 1 you will be reimbursed for your time and effort. You will then be invited to participate in the additional phases of this research study which are described next.

PHASE 2 – *Phone screening and demographic session (15-20 minutes).*

- If you are interested in participating further in the study, we will conduct a phone screening session with you. This will involve a phone call from us during which you will need to answer demographic questions, and we will also ask about any history of head injury, the presence of any metal objects in the body, etc., to determine your suitability for the MRI scan. We will also be asking questions about your health which will involve questions relating to medical history, past and current medications, and drug and alcohol use. This is to ensure that you are fit and suitable to participate in this research study. Once the screening has been passed, we will arrange a time for the next session that is most suitable for you to attend the brain scanning session.

PHASE 3 – *Brain scan (MRI) assessment and biological samples (3-4 hours).*

- For the *MRI scan*, you will be screened again by a radiographer to ensure you are ready to enter the MRI scanner. You will be in the scanner for approximately 40 minutes during which we will talk to you throughout the session via a microphone and you can also communicate with us. During the scan, you will be asked to complete two computerised tasks and you will be given a button box in your hands to make your responses – this will be similar to playing a computer game, only this time you will be doing this inside a MRI scanner. Both tasks involve viewing pictures of human faces with different emotional expressions. You will have a chance to practice these tasks prior to entering the scanner to make sure you are familiar with how it all works. For more information, you can also visit the website for Swinburne's neuroimaging facility: http://www.swinburne.edu.au/lss/bpsyc/facilities.html. The MRI scan session will be conducted at **The Brain and Psychological Sciences Research Centre (Swinburne University of Technology, Burwood Hwy, Hawthorn)**; see map at end of document.

- *Biological samples* will be collected before or after the MRI scan. This may include a blood sample, several saliva samples, and collecting cells (buccal cells) from the inside of your cheeks using a cotton bud. These samples will be used to measure the concentration levels of key hormones (oxytocin and vasopressin as well as the stress-related hormone, cortisol) in your body so that we can see how these hormones relate to your thinking processes and brain activity. These samples will be collected at the pathology centre at the **Royal Children's Hospital, Melbourne** (blood, saliva, cheek cell samples) and at **the School of Psychology on the Melbourne campus of ACU (located at 11-14 Young Street, Fitzroy)** (saliva samples).

At the end of phase 2 and 3, you will be reimbursed for your time and effort accordingly.

Are there any risks associated with participating in this study?

The potential risks associated with this study are minimal. However, it is possible that you may experience some discomfort during the following procedures:

i. *7-day ambulatory assessment with mobile phone app*: Some people may find responding to the smartphone surveys somewhat disruptive. However, we have experience in this method of research and do not anticipate any distress. Should you experience any distress as a result of your participation in this study, please contact Dr Izelle Labuschagne (e-mail: izelle.labuschagne@acu.edu.au; Ph: 03 9953 3816) or Dr Peter Koval (e-mail: peter.koval@acu.edu.au; Ph: 03 9230 8088) who will refer you to an independent counselling service if required.

ii. *MRI scan:* The MRI procedure does not involve exposure to any ionizing radiation, and there are no known health risks associated with the MRI procedure. Some discomfort may be felt during scanning due to the small cavity of the MRI scanner which could restrict your body movements, and also due to the noise from the MRI scanner. To minimise and manage any risks, you will be thoroughly screened for MRI clearance prior to participating by both the research staff and the MRI radiographer. This will involve questioning you about metal implants or accidents whereby metal may have become

lodged in the body, and about potential pregnancies. Physical discomfort inside the scanner will be reduced by cushioning and earplugs (standard procedure for MRI facilities). You will be able to communicate with the radiographer and the researcher at all times during scanning and you will also have access to a 'panic' button which you may press at any stage to stop the scanner if you are not comfortable to continue.

iii. *Collection of blood, saliva, and cells from the cheeks:* We anticipate that the collection of these samples will produce minimal risk. The blood will be collected by an experienced nurse located at the Royal Children's Hospital, which means they are experienced in collecting blood from vulnerable people such as children. The saliva samples will be collected by the participants themselves into small tubes, guided by the researcher. The collection of buccal cells from the inside of the cheeks involves using a small cotton swab and rubbing this against the inside of your cheek for about 5-10 s. These cells provides us with an excellent source to measure DNA that will be used in our genetic analyses. All these procedures are common to research involving human adults and children and are considered safe and harmless. However, to ensure we are not causing any distress to you or make you feel uncomfortable, we will carefully explain the procedures and will allow you to ask any questions. In the event that you are not comfortable with the procedures, we will not continue with the collection and there will be no consequence to you or your participation in the study.

What if I become distressed during my participation?

Your behaviour will be closely monitored at all times to ensure you are comfortable with all the procedures involved. Please note that there are no consequences to letting us know that you are uncomfortable about any of the procedures and/or that you would like to discontinue or pause the session. See also below *Can I withdraw from the study?* If at any time during the project you do become distressed, we will provide you with our full support to accommodate any needs. We have a clear risk plan in place where we will ensure that you are supported. With permission from you, we will have access to psychologists and senior researchers who can talk to you. We also have a detailed list of services that you could contact for further support. We can also refer you to our local ACU Melbourne Psychology and Counselling Clinic for which we will provide details. Finally, we would be happy to also communicate any concerns directly to your regular health professional (e.g., GP or mental health specialist) or a close member whether it be a family member or friend.

How much time will the study take?

Phase 1 will involve an introductory lab session of approximately 2 hours. The second lab session will take approximately 120 minutes. Each smartphone survey will take 1-2 minutes to complete and there will be a maximum of 70 surveys over the course of 1 week. Therefore, the total time commitment during the 7-day assessment is approximately 4-5 hours.

Phase 2 and 3 of this research study will require a phone screening session of approximately 20 minutes during a convenient time and may be done over the phone. If you are suitable for the MRI scan, the MRI session will take approximately 2 hours which includes the 40 min MRI scan, as well 1 hour for collecting biological samples, allowing for time between places which will be arranged.

What are the benefits of the research study?

This research study will help us answer important scientific questions regarding the role of biological and thinking processes in the daily lives of individuals with SAD. While there are no direct benefits to individuals who choose to participate in this research, the findings from this study have significant implications for those suffering from social anxiety and may even further implicate other psychiatric disorders given overlap in symptoms of anxiety and emotion processing. If you decide to take part you have an opportunity to use the latest cutting-edge tools for monitoring emotions in daily life and get first-hand experience of how psychology research is conducted. Your time and efforts will be reimbursed to a maximum amount of $100

for your time involvement in phase 1 of the study. Participation in Phase 2 and 3 will also be reimbursed a further $100 in the form of a gift card. For those entering Phase 4 of the study, you may experience some benefit in the form of alleviating your social anxiety symptoms and understanding more about social anxiety in general

Can I withdraw from the study?
Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate, you can withdraw from the study at any time without adverse consequences. If you are currently getting treatment, there will also be no consequences to your treatment in the future if you choose to withdraw. If you withdraw early, you will be reimbursed pro rata based on your time investment in the study.

Will anyone else know the results of the study?
The results of the study will be published in international journals and presented at local and international conferences. However, all publications will only involve group-level results and no individual participant will be identifiable based on these findings. All data will be deidentified at the point of collection, and only a unique ID code will be used to link the various data sources for each participant. Data in a digital form will be stored on a secure, password protected computer network accessible only by the researchers. If data are shared with other researchers, they will be in an anonymous de-identified format (only ID codes will be used).

Will I be able to find out the results of the study?
Participants who are interested in receiving a summary of the study's findings may contact the researchers (see contact details below) at the end of the study. A report of the aggregated results will be made available to participants upon request approximately 2-3 months following the completion of the study. We will provide all the participants with a copy of the study report or publication upon request.

Who do I contact if I have questions about the study?
Any questions regarding this study can be directed to the main researchers: Dr Izelle Labuschagne (ph: 03 9953 3816 or email: izelle.labuschagne@acu.edu.au and Ms Caitlin Grace: caitlin.grace2@myacu.edu.au

What if I have a complaint or any concerns?
The study has been reviewed by the Human Research Ethics Committee at Australian Catholic University (HREC 2016-217H). If you have any complaints or concerns about the conduct of the study, you may write to the Manager of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).
Manager, Ethics
c/o Office of the Deputy Vice Chancellor (Research)
Australian Catholic University
North Sydney Campus
PO Box 968
NORTH SYDNEY, NSW 2059
Ph.: 02 9739 2519
Fax: 02 9739 2870
Email: resethics.manager@acu.edu.au

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

I want to participate! How do I sign up?
If you are interested in the study, you can contact the researchers by phone or email and they will then arrange a time to do the initial phone call. You will be sent a copy of the consent form and information document which you will need to read and sign. If you have not signed the

consent form prior to attending the lab session, you may do so at the beginning of the lab session.

Yours sincerely,
Primary Contacts:

Ms Caitlin Grace
E-mail: caitlin.grace4@myacu.edu.au
Dr Izelle Labuschagne
E-mail: izelle.labuschagne@acu.edu.au
Ph: (03) 9953 3816

MAPS

The Mary Glowrey Building, Level 3 (Building number 420; or the red arrow/number 1 on map below)
Australian Catholic University
115 Victoria Parade, Fitzroy, Melbourne

Appendix A – 4. Consent Form

CONSENT FORM
*Copy for **Researcher***

TITLE OF PROJECT: **Daily Emotional Functioning in Social Anxiety Disorder**.....

PRINCIPAL INVESTIGATORS: Dr Izelle Labuschagne; Dr Peter Koval; Dr Gill Terrett; Prof Peter Rendell .....

STUDENT RESEARCHERS: Ms Caitlin Grace .....

I ..... *(the participant)* have read (*or, where appropriate, have had read to me)* and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in the selected phases of this study, which will involve:

Current:

- ☐ **Phase 1:** An 8 day testing cycle involving; (a) 2 Lab sessions including an initial lab session (including completion of questionnaires and providing samples such as saliva and cells form my cheek), and a second Lab session including a social experiment, and (b) a 7-day ambulatory monitoring involving completion of 10 brief smartphone surveys per day and 3 days of at home collection of saliva. **(Please tick the box if you agree).**

Secondary (at a later date):

- ☐ **Phase 2 and 3:** approximately 2 hours of completing questionnaires and tasks, including a 40-min MRI scan and providing some samples (of blood and some cells from my cheek) **(Please tick the box if you have not already participated in phase 2 and 3 and would like to be contacted to participate at a later date. Do not tick the box if you would only like to participate in Phase 1).**

I understand some of the questionnaire items relate to unpleasant events, emotions, and thoughts and may therefore be distressing to some people. If I experience any distress as a result of my participation in this study, I understand that I may contact Prof John Gleeson, a registered clinical psychologist, on (03)9953 3108 for appropriate advice and support.

I realize that I can withdraw my consent at any time (without adverse consequences, and I will be reimbursed for my time pro rata). I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way. I agree to participate in this research realising that information gathered will remain confidential and secure except when it is required by law, and/or failure to disclose the information would place myself or another person at risk. I agree for my data, which will not be identifiable, to be used in future studies.

NAME OF PARTICIPANT: .....

SIGNATURE .....

DATE .....

SIGNATURE OF PRINCIPAL INVESTIGATOR (or SUPERVISOR):
DATE:.....
SIGNATURE OF STUDENT RESEARCHER:
DATE:.....

☐ **I agree to be contacted for participation in future ACU research studies (please tick box)**

CONSENT FORM
*Copy for **Participant***

TITLE OF PROJECT: **Daily Emotional Functioning in Social Anxiety Disorder**.....

PRINCIPAL INVESTIGATORS: Dr Izelle Labuschagne; Dr Peter Koval; Dr Gill Terrett; Prof Peter Rendell .....

STUDENT RESEARCHERS: Ms Caitlin Grace .....

I ..... *(the participant)* have read (*or, where appropriate, have had read to me)* and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in the selected phases of this study, which will involve:

Current:

- ☐ **Phase 1:** An 8 day testing cycle involving; (a) 2 Lab sessions including an initial lab session (including completion of questionnaires and providing samples such as saliva and cells form my cheek), and a second Lab session including a social experiment, and (b) a 7-day ambulatory monitoring involving completion of 10 brief smartphone surveys per day and 3 days of at home collection of saliva. **(Please tick the box if you agree).**

Secondary (at a later date):

- ☐ **Phase 2 and 3:** approximately 2 hours of completing questionnaires and tasks, including a 40-min MRI scan and providing some samples (of blood and some cells from my cheek) **(Please tick the box if you have not already participated in phase 2 and 3 and would like to be contacted to participate at a later date. Do not tick the box if you would only like to participate in Phase 1).**

I understand some of the questionnaire items relate to unpleasant events, emotions, and thoughts and may therefore be distressing to some people. If I experience any distress as a result of my participation in this study, I understand that I may contact Prof John Gleeson, a registered clinical psychologist, on (03)9953 3108 for appropriate advice and support.

I realize that I can withdraw my consent at any time (without adverse consequences, and I will be reimbursed for my time pro rata). I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way. I agree to participate in this research realising that information gathered will remain confidential and secure except when it is required by law, and/or failure to disclose the information would place myself or another person at risk. I agree for my data, which will not be identifiable, to be used in future studies.

NAME OF PARTICIPANT: .....

SIGNATURE .....

DATE .....

SIGNATURE OF PRINCIPAL INVESTIGATOR (or SUPERVISOR):
DATE:.....
SIGNATURE OF STUDENT RESEARCHER:
DATE:.....

☐ **I agree to be contacted for participation in future ACU research studies (please tick box)**

Appendix A – 5. Participant saliva record booklet

Saliva Collection Kit

Your Cortisol Collection Handbook

An instruction guide and record book for the collection of 3 days of Saliva Samples across your participation in the Daily Emotional Functioning Study.

Your 3 Day Saliva Collection Kit

Firstly, Thank you for your participation in this study!

As a part of your participation you will be collecting 3 full days' worth of Saliva Samples while at home going about your daily life.

Why Saliva? Well, simple saliva can tell us a great deal about your bodies functioning in your daily life – in particular we want to take a look at Cortisol (what some people call "the stress hormone") and how it changes in your body across the day. We'll be looking at Cortisol in two types of contexts:

1. Your Cortisol Awakening Response or "CAR" – a 1Hr period immediately after waking

2. Your Cortisol across the entire day – from 8am to 10pm.

Your overall participation in this study spans across 8 days, and you will be using your collection kit to take Saliva Samples on Days 2, 4 and 6 at specific time points. You will be collecting two lots of samples on these days for these two very different cortisol responses (your CAR cortisol and your Across the Day Cortisol)

This saliva sampling is crucial to our study, and it is very important that together we can be as accurate as possible and be careful to track the specific details of your sampling. It is integral to our study that the correct number of samples be collected at the specified times. This will help us to paint as accurate a picture of the stress hormone 'Cortisol' in your body across your daily life. We understand it is not always possible to be spot on with the times or sometimes things don't go exactly as planned, so if a time point is missed or something doesn't go quite as planned we ask that you simply record some information down for us in your notes section, including the actual time of sampling.

You will have one kit clearly labelled for each of the 3 collection days two cycles of collection (your CAR cycle and Across the Day cycle) –These will come in a handy container you may take around with you or leave in your fridge. Inside the container, there will be a number of vials. These will all be clearly labelled with the day and time of each sample.

It is VERY important the correct saliva samples go into the correct vials.

Your researcher will take the time to go over all of the details of collection with you – be sure to ask any questions, and more importantly – should you have any questions or concerns while participating, your research will happily help you out.

We just want to get this right – so we are happy for you to contact us whenever you need!

Happy Collecting!

How Do You Collect the Saliva? That's Easy…

a. Peel back protective package and remove the SOS (that's the chewy cotton bud thing).
b. Remove SOS from outer packaging and place under the tongue.
c. Keep SOS in the mouth, in the same location (snug under your tongue) for 1-2 minutes (around 1 minute should do it – just make sure it's nice and juicy).
d. Remove cap from purple storage tube (there's two parts to this purple tube, the smaller inner tube and a large tube which surrounds the inner tube).
e. Remove the SOS from your mouth and place into the tube insert ("swab basket" – this is the smaller inner tube) of the swab storage tube (SST).
f. Recap SST tightly. *Note:* Do not throw away any parts of the tube assembly! (We need that for later on).

One last thing! Here's some more really important information about your CAR SALIVA COLLECTION….

1. If you happen to wake up at 8am – or set an alarm for 8am (e.g. the same time as your full day of Saliva collection starts) and this coincides with your CAR sampling, then you'll need to take TWO SAMPLES at 8am (your 1st CAR sample, and your 1st Daily Sample)
2. DURING your 1HR CAR COLLECTION – it's very important you don't:

   - DRINK CAFFEINE/CAFFEINATED BEVERAGES

   - DRINK SUGARY DRINKS

   - EAT BREAKFAST/ANY FOOD (just for the hour – promise!)

   - EXERCISE – NO PHYSICAL EXERCISE OR EXERTING YOURSELF

3. But most importantly…. If any of the above occurs in that first hour, or if anything goes haywire – just let us know. Jot it down in the notes section of your booklet or scribble it somewhere for us. We understand sometimes these things don't go to plan, and the best way for us to manage it is to be aware of what happened. This doesn't affect your participation or how much you're reimbursed – it just helps us take care of our data and we researchers really love our data!

Key Pointers for Collection

How Many Samples Each Day?

- CAR Samples in the first hour you wake up
- Samples across the day from 8am to 10pm

Total? 11 Samples each day for the 3 Days – Your kit should end up with 33 samples by close of Day 6!

Storing Your Samples

You can keep the whole sample box in the fridge – that's what is best for the samples. If you like – you can take your days' worth of samples in a snap lock bag when you head out for the day and simply transfer them back to the box in the fridge when you return home. Just make sure your samples are going into the correctly labelled vials!

Example Daily Record Sheet

Your Daily Record – Day 2… Let's get started!

DATE:		
What time did you first see Daylight? _____ : _____		
TESTING DAY	**Day 2 CAR** **All 4 samples within 1HR After Waking!**	**Day 2**
	☐ **Awakening – (0 Mins – right away!)** LABEL: **BC – CAR0** **300317** **DEV400** Record Time: _____	**Collecting 7 Samples across the day alongside your 4 CAR Samples**
	☐ **CAR 2 – (+30 Mins after waking)** LABEL: **BC – CAR30** **300317** **DEV400** Record Time: _____	**SAMPLE Number and Time of Collection**: **Sample 1** ☐ **8:00am** Record Time: _____ — **LABEL**: **BC – S1 300317 0800 DEV400**
	☐ **CAR 3 – (+45 Mins after waking)** LABEL: **BC – CAR45** **300317** **DEV400** Record Time: _____	**Sample 2** ☐ **10:00am** Record Time: _____ — **BC – S2 300317 1000 DEV400**
	☐ **CAR 4 – (+60 Mins after waking)** LABEL: **BC – CAR60** **300317** **DEV400** Record Time: _____	**Sample 3** ☐ **12:00pm** Record Time: _____ — **BC – S3 300317 1200 DEV400**
		Sample 4 ☐ **15:00 (3pm)** Record Time: _____ — **BC – S4 300317 1500 DEV400**
		Sample 5 ☐ **18:00 (6pm)** Record Time: _____ — **BC – S5 300317 1800 DEV400**
		Sample 6 ☐ **20:00 (8pm)** Record Time: _____ — **BC – S6 300317 2000 DEV400**
		Sample 7 ☐ **22:00 (10pm)** Record Time: _____ — **BC – S7 300317 2200 DEV400**

Collection Notes:
Tell us how the day went - Any Changes or Issues?

Appendix B – Ethics Approval, Modification and Extension Correspondence

Appendix B – 1. Original Ethics Approval

Principal Investigator: Dr Izelle Labuschagne
Co-Investigator: Dr Skye McLennan, Dr Darren Hocking, Prof Peter Rendell, Dr Peter Koval
Student Researcher: Caitlin Grace (HDR Student)
Research Assistant: Ms Rachel Pelly

Ethics Register Number: 2016-217H
Project Title: Daily emotional functioning in Social Anxiety Disorder
Risk Level: Low Risk
Date Approved: 24/11/2016
Ethics Clearance End Date: 31/12/2017

This email is to advise that your application has been reviewed by the Australian Catholic University's Human Research Ethics Committee and confirmed as meeting the requirements of the National Statement on Ethical Conduct in Human Research.
The data collection of your project has received ethical clearance but the decision and authority to commence may be dependent on factors beyond the remit of the ethics review process and approval is subject to ratification at the next available Committee meeting. The Chief Investigator is responsible for ensuring that outstanding permission letters are obtained, interview/survey questions, if relevant, and a copy forwarded to ACU HREC before any data collection can occur. Failure to provide outstanding documents to the ACU HREC before data collection commences is in breach of the National Statement on Ethical Conduct in Human Research and the Australian Code for the Responsible Conduct of Research. Further, this approval is only valid as long as approved procedures are followed.

If your project is a Clinical Trial, you are required to register it in a publicly accessible trials registry prior to enrolment of the first participant (e.g. Australian New Zealand Clinical Trials Registry http://www.anzctr.org.au/) as a condition of ethics approval.

If you require a formal approval certificate, please respond via reply email and one will be issued.

Researchers who fail to submit a progress report may have their ethical clearance revoked and/or the ethical clearances of other projects suspended. When your project has been completed a progress/final report form must be submitted. The information researchers provide on the security of records, compliance with approval consent procedures and documentation and responses to special conditions is reported to the NHMRC on an annual basis. In accordance with NHMRC the ACU HREC may undertake annual audits of any projects considered to be of more than low risk.

It is the Principal Investigators / Supervisors responsibility to ensure that:
1. All serious and unexpected adverse events should be reported to the HREC with 72 hours.
2. Any changes to the protocol must be reviewed by the HREC by submitting a Modification/Change to Protocol Form prior to the research commencing or continuing.
http://research.acu.edu.au/researcher-support/integrity-andethics/
3. Progress reports are to be submitted on an annual basis.
http://research.acu.edu.au/researcher-support/integrityand-ethics/
4. All research participants are to be provided with a Participant Information Letter and consent form, unless otherwise agreed by the Committee.
5. Protocols can be extended for a maximum of five (5) years after which a new application must be submitted (The five year limit on renewal of approvals allows the Committee to fully re-review research in an environment where legislation, guidelines and requirements are continually changing, for example, new child protection and privacy laws).
Researchers must immediately report to HREC any matter that might affect the ethical acceptability of the protocol e.g.: changes to protocols or unforeseen circumstances or adverse effects on participants.

Please do not hesitate to contact the office if you have any queries.

Kind regards,
Kylie Pashley on behalf of ACU HREC Chair, Dr Nadia Crittenden
Ethics Officer | Research Services
Office of the Deputy Vice Chancellor (Research) Australian Catholic University

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Appendix B – 2. Ethics Modification Approval Notifications (Earliest to Latest)

Ethics Register Number: 2016-217H
Project Title: Daily emotional functioning in Social Anxiety Disorder
End Date: 31/12/2017

Thank you for submitting the request to modify form for the above project.
The Chair of the Human Research Ethics Committee has approved the following modification(s):

1. Addition of Trier Social Stress Test (health controls only).
2. Additional saliva samples as outlined.
3. Update questions in the Ecological Momentary Assessment questionnaire.
4. New assessments: Rosenberg Self-esteem Scale, The Brief Fear of Negative Evaluation Scale, The Fear of Positive Evaluation Scale, The Childhood Trauma Questionnaire, the MINI-Internal Neuropsychiatric Interview. NOTE: An experienced counsellor must be available at any and all times that The Childhood Trauma Questionnaire is administered to participants.
5. Additional handout
6. Removal of hair samples.
7. Addition of Assoc Prof Leah Brennan and Ms Laura Payne (psychology student) to the protocol.

We wish you well in this ongoing research project.
Kind regards,
Ms Kylie Pashley
Research Ethics Officer | Office of the Deputy Vice-Chancellor (Research)
Australian Catholic University
T: 02 9739 2646 E: res.ethics@acu.edu.au

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Ethics Register Number: 2016-217H
Project Title: 2016-217H
End Date: 31/12/2018
Thank you for submitting the request to modify form for the above project.
The Chair of the Human Research Ethics Committee has approved the following modification(s):

1. An updated version of the participant information letter (V4), advertising poster (V2) and consent form (V2) to include already approved study modifications.
2. Increase in participant reimbursement to accommodate an increase in participant effort.

We wish you well in this ongoing research project.
Kind regards,
Ms Pratigya Pozniak

Research Ethics Officer | Office of the Deputy Vice-Chancellor (Research)
Australian Catholic University
T: 02 9739 2646 E: res.ethics@acu.edu.au

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Ethics Register Number: 2016-217H
Project Title: Daily emotional functioning in Social Anxiety Disorder
End Date: 31/12/2018
Thank you for submitting the request to modify form for the above project. The Chair of the Human Research Ethics Committee has approved the following modification(s):

1. Patients with Social Anxiety Disorder (SAD) to also now complete the Trier Social Stress Test.
2. Adding the self-report questionnaire: Attachment Style Questionnaire (ASQ)
3. Addition of personnel: Dr Katie Bunch and Dr Sarah Mitchell

We wish you well in this ongoing research project.
Kind regards,
Ms Pratigya Pozniak
Research Ethics Officer | Office of the Deputy Vice-Chancellor (Research)
Australian Catholic University
T: 02 9739 2646 E: res.ethics@acu.edu.au

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Ethics Register Number: 2016-217H
Project Title: Daily emotional functioning in Social Anxiety Disorder
End Date: 31/12/2018
Thank you for submitting the request to modify form for the above project. The Chair of the Human Research Ethics Committee has approved the following modification(s):

1. Update on PhD student clinical qualifications
2. Removal of the requirement to have a senior psychologist available on call for SAD participants completing the Trier Social Stress Test (TSST). The PhD student will now undertake this initial role whilst still having the option to refer participants to clinical psychologists with AHPRA registration should it be required.

We wish you well in this ongoing research project.
Kind regards,
Ms Kylie Pashley
Research Ethics Officer | Office of the Deputy Vice-Chancellor (Research)
Australian Catholic University
T: 02 9739 2646 E: res.ethics@acu.edu.au

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Appendix B – 3. Extension Request Approval from HREC

End of 2018.

Ethics Register Number: 2016-217H

Project Title: Daily emotional functioning in Social Anxiety Disorder

Data Collection Date Extended: 31/12/2018

Thank you for returning the Ethics Progress Report for your project.

The Deputy Chair of the Human Research Ethics Committee has approved your request to extend the project. The new expiry date for the project is the 31/12/2018.

We wish you well in this ongoing project.

Kind regards,

Ms Pratigya Pozniak

Research Ethics Officer | Office of the Deputy Vice-Chancellor (Research)

Australian Catholic University

T: 02 9739 2646 E: res.ethics@acu.edu.au

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End of 2019.

Ethics Register Number: 2016-217H

Project Title: Daily emotional functioning in Social Anxiety Disorder

Data Collection Date Extended: 31/12/2019

Thank you for returning the Ethics Progress Report for your project.

The Deputy Chair of the Human Research Ethics Committee has approved your request to extend the project.

The new expiry date for the project is the 31/12/2019.

We wish you well in this ongoing project.

Kind regards,

Ms Kylie Pashley

Research Ethics Officer | Office of the Deputy Vice-Chancellor (Research)

on behalf of ACU HREC Chair, Assoc Prof. Michael Baker

Australian Catholic University

T: 02 9739 2646 E: res.ethics@acu.edu.au

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Appendix B – 4. Approval to Reproduce SAD Criteria from APA

Dear Caitlin Grace,
Permission is granted for use of the material as outlined in the request below for your dissertation only.
Permission is granted under the following conditions:

- Material must be reproduced without modification, with the exception of style and format changes
- Permission is nonexclusive and limited to this one-time use
- Use is limited to English language only
- Permission must be requested for additional uses (including subsequent editions, revisions, and any electronic use)
- No commercial use is granted

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