School of Population Health

Does Heart Rate Variability Better Differentiate Individuals With and Without an Anxiety Disorder at Rest or During Stress?

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007) – updated March 2014. The proposed research study received human research ethics approval from the Curtin University Human Research Ethics Committee (EC00262), Approval Number HRE2020-0159

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List of abbreviations

ANS	Autonomic Nervous System
CBT	Cognitive Behaviour Therapy
CI	Confidence Interval
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5th Edition
EM	Expectation Maximisation
ES	Effect Size
GAD	Generalised Anxiety Disorder
GUTS	Generalised Unsafety Theory of Stress
HF	High Frequency
HRV	Heart Rate variability
LF	Low Frequency
M	Mean
MSD	Mean of Successive RR Differences
n	Sample Size
PD	Panic Disorder
PNS	Parasympathetic Nervous System
RDoC	Research Domain Criteria
RMSSD	Root Mean Square of Successive Differences Between Heartbeats
SAD	Social Anxiety Disorder
SCID	Structured Clinical Interview for DSM-5
SD	Standard Deviation
SE	Standard Error
SIAS	Social Interaction Anxiety Scale
SNS	Sympathetic Nervous System
SPS	Social Phobia Scale
TD	Time Domain
TSST	Trier Social Stress Test

Chapter 1: Overview

Introduction

The following general introduction provides an overview of specific physiological processes that have been examined in relation to anxiety disorders, namely heart rate variability. An overview of key HRV measures is provided with briefly consideration of how these have been examined in relation to anxiety disorders, and outstanding research questions that form the basis of investigations. As the two papers presented in the thesis provide further specific background detail in their respective introductions, the level of detail in this introduction is relatively brief to avoid unnecessary repetition.

Anxiety disorders are one of the most prevalent class of mental disorders, with 14.4% of the Australian population meeting criteria for diagnosis in their lifetime (Australian Bureau of Statistics [ABS], 2007). Anxiety disorders can have a debilitating effect on an individual's quality of life, with many areas affected including education and work, social relationships, and physical health, depending on the specific disorder (Olatunji et al., 2007). Anxiety disorders have traditionally been examined with a focus on cognitive or behavioural symptoms (Baldwin et al., 2013; Bandelow et al., 2008; National Institute for Health and Clinical Experience [NICE], 2013). However, exploring other modalities of the disorders, such as physiological influences, can provide a more complete understanding, and potentially also inform future treatment avenues.

Typically, first line treatment for many anxiety disorders is a combination of pharmacological intervention and psychotherapy, namely, cognitive behavioural therapy (CBT; Bandelow et al., 2017). CBT focuses on cognitive and behavioural aspects of a disorder, while placing less of an emphasis on the role of psychophysiology in the aetiology and maintenance of a disorder (Hofmann, 2007). Given that cognitive, behavioural, emotional and physical symptoms of anxiety are inextricably linked, cognitive behavioural approaches to treatment likely influence psychophysiology, and therefore treating symptoms through one modality is likely to have an effect on another (Engel, 1980). Thus, gaining a better understanding of the physiological processes associated with anxiety can complement cognitive behavioural models of the disorder, as well as opening additional avenues for treatment targets.

Research examining biological influences on anxiety and their implications for treatment has been rapidly developing (Spence & Rapee, 2016). Typically, the effectiveness of treatments for anxiety are measured by assessing the impact of a therapeutic intervention such as CBT on self-report or clinician-rated change (NICE, 2013). While placing primary emphasis on individual perceptions of symptom severity, this approach deemphasises the role of physiological processes that may moderate or mediate treatment response and maintenance of an anxiety disorder (Hyett

et al., 2018). A better understanding of the way physiological processes relate to self-report symptom severity, and how such processes respond to psychological intervention, will lead to a more complete understanding of anxiety.

A particularly influential model of anxiety-physiology interactions is provided by Porges' (2003) polyvagal theory, in which he outlines a crucial relationship between functioning of the autonomic nervous system (ANS) and an individual's ability to regulate social and emotional behaviour. The theory posits that the physiological state of an individual limits their range of behaviour and psychological experience, and that the evolution of the nervous system is linked to affective experience, emotional expression, facial gestures, vocal communication, and social behaviours (Porges, 2003). Porges suggests that a key aspect of many anxiety disorders is a reduced ability to control maladaptive autonomic processes, as individuals may be unable to effectively identify safe environments, leading to a constant state of heightened arousal. Thus, many psychiatric disorders of mood and affective experience, such as anxiety disorders, are inextricably linked with autonomic control (Porges, 2003). Similarly, in their neurovisceral integration model, Thayer and Lane (2000) posit that the neural networks responsible for autonomic, emotional, and cognitive regulation, are also associated with control of cardiac autonomic activity. Dysfunction in these networks may lead to problems with autonomic inhibition, a key characteristic of many anxiety disorders (Thayer & Lane, 2000).

Many indices of ANS rely on either electrodermal (i.e. sweat response) or cardiovascular (i.e. circulatory systems) responses (Mauss & Robinson, 2009). These measures are mainly affected by the parasympathetic nervous system (PNS) and sympathetic nervous system (SNS), which are indices of ANS regulation (i.e. rest and digest) and arousal (i.e. fight or flight), respectively (Appelhans & Luecken, 2006). Heart rate variability - the subtle fluctuations of intervals between heart beats, usually measured in milliseconds - is a commonly used measure of ANS functioning. HRV can be measured through either time-domain or frequency-domain indices (Shaffer & Ginsberg, 2017). Time-domain indices quantify the amount of variability between heartbeats over a period of time, while frequency-domain indices examine power, the signal energy of heartbeats at a number of different frequency bands (Shaffer & Ginsberg, 2017). Different indices of HRV reflect relative activation of parasympathetic and sympathetic activity.

There are a number of different frequency-domain measures of HRV that are predominantly influenced by the PNS, including absolute power of the high-frequency band (Shaffer & Ginsberg, 2017). High frequency power is commonly used within the literature, and is a measure of the signal strength of the high frequency band, which occurs at 0.15-0.4 Hz and is influenced by breathing at 9-24 beats per minute (Shaffer & Ginsberg, 2017). High frequency power is commonly used as an index

of PNS functioning (Shaffer & Ginsberg, 2017). One time-domain predominantly parasympathetic measure of HRV is the root mean square of successive differences between normal heartbeats (RMSSD; National Institute of Mental Health (NIMH) ,2016; Shaffer & Ginsberg, 2017). RMSSD is the most common time-domain measure of HRV, and reflects the beat-to-beat variance in heart rate (Shaffer & Ginsberg, 2017). Absolute power of the low frequency band, another commonly assessed frequency domain index of HRV, is a measure of the signal strength of the low frequency band, occurring in the range of 0.04-0.15 Hz (Shaffer & Ginsberg, 2017). Low frequency power is often used as an index of SNS functioning (Houle & Billman, 1999). Another index of HRV is respiratory sinus arrhythmia (RSA). RSA is a measure of fluctuations in heart rate in phase with respiration (Grossman & Taylor, 2007). While there is a relationship between RSA and autonomic activity, RSA is also influenced by breathing rate (Shaffer & Ginsberg, 2017). That is to say, respiration itself can cause large shifts in RSA magnitude, independently of vagal tone itself (Shaffer & Ginsberg, 2017).

Such HRV indices have previously been used as a sensitive biomarker of emotional regulation (Thayer & Lane, 2000). A lower level of HRV is associated with a reduced ability to control maladaptive autonomic response in the face of perceived threats and stress, whereas a higher level of HRV is associated with better adaption to stimuli and cognitive flexibility (Quintana et al., 2016). Thus, according to the polyvagal theory, individuals with an anxiety disorder will typically display lower levels of HRV, as they are less able to regulate affective experience in the face of threatening or stressful situations (Porges, 1995). Indeed, previous literature has shown that individuals with an anxiety disorder display significantly lower levels of HRV than control participants, at least during rest (while no stress is taking place; Chalmers et al., 2014). There has, however, been a growing interesting in how stress itself influences HRV in individuals with anxiety disorders. Acute stress plays an important role in many anxiety disorder (e.g. social interactions in SAD; Beck et al., 2005), so by examining physiology during stress, we can deduce whether physiology is further impacted, or indeed impacted at all, during acute stressful scenarios, a key contributing factor to many anxiety disorders.

Structure and Aims of the Thesis

This thesis aims to examine HRV in individuals with any Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5, American Psychiatric Association [APA], 2013) anxiety disorder, as well as healthy controls. While there is a strong literature base supporting lower HRV in individuals with an anxiety disorder at rest, literature is still mixed surrounding the influence of a stress task on HRV in anxious participants. Thus, this study will examine HRV both at rest (baseline), and during disorder-relevant stress tasks. In order to address the yet un-answered question about whether HRV better

differentiates between anxious and control participants at rest or during disorder relevant stress, Chapter two (Study 1) reports a meta-analysis that compared HRV in clinically anxious and non-anxious samples, both at rest and during a disorder-relevant stress task. Having identified the examination of HRV under rest-stress as a specifically under-researched area in social anxiety disorder, Chapter three (study 2) then considers whether HRV better differentiates between socially anxious individuals and controls at rest or during social stress. Finally, chapter four contains a general discussion synthesising the results of the two studies, before outlining limitations, future research directions, and conclusions.

Chapter 2 - Meta-analysis

Abstract

Anxiety disorders are typically characterised by lower levels of heart rate variability (HRV) at rest (while no stress is taking place), however there is mixed literature surrounding the nature of HRV in individuals with anxiety disorders while they are under disorder-relevant stress. This meta-analysis synthesised data from studies that examined HRV in individuals with and without a DSM-5 categorised anxiety disorder, while at rest and during a disorder-relevant stress task. The analysis aimed to determine whether HRV differentiated between anxious and control participants at rest, whether this difference changed during disorder relevant stress, and how the stress tasks themselves would influence HRV within the two groups. Meta-analyses were based on 9 articles, with 202 clinical participants and 206 controls. Results found that HRV was consistently lower in anxious individuals, both at rest and during stress, but that the stress tasks themselves did not influence HRV in either group. Consistent with theoretical models such as the polyvagal theory, the results indicate that trait-level lowered parasympathetic regulation, as indexed by HRV, is a key characteristic of many anxiety disorders.

A meta-analysis of heart rate variability at rest and during disorder-relevant stress in clinical anxiety

Psychiatric disorders have traditionally been diagnosed from clinician and client-reported behaviours and associated features (Kozak & Cuthbert, 2016). The classification of these disorders can be seen as theoretical constructs created through stipulation surrounding observed behaviours and symptoms, constructed and refined over successive iterations (Kozak & Cuthbert, 2016). While these traditional categories of psychiatric disorders afford some simplicity that is helpful for communication and decision-making, they do not allow sharp boundaries between what classifies one mental disorder vs. another, and do not account for differential symptom severity (Kozak & Cuthbert, 2016). The NIMH Research Domain Criteria (RDoC) initiative is an effort to formulate a better framework for psychopathology research by adopting a dimensional approach, and integrating elements of psychology and biology (Kozak & Cuthbert, 2016). By taking a more dimensional approach to psychopathology, 'cut points' can be established, allowing disorders to be categorised precisely into mild, moderate and severe symptom severity, thus more validly reflecting dimensional constructs (Kozak & Cuthbert, 2016). The RDoC initiative advocates a wide range of measures in psychotherapy research, integrating self-report and biological measures, to form a more comprehensive view of psychopathology that includes multiple empirically tested accurate measures of a construct (such as anxiety; Kozak & Cuthbert, 2016). One key biological measure that the RDoC initiative recommends for indexing arousal and regulatory systems is that of HRV (NIMH, 2016).

The polyvagal theory posits that a key aspect of many anxiety disorders is a reduced ability to control maladaptive autonomic processes (Porges, 2003). Consistent with this, there is direct evidence for systematic differences in indicators of autonomic arousal between individuals with and without an anxiety disorder. Chalmers et al.'s (2014) systematic review examined HRV measured at baseline (during rest) in individuals with and without an anxiety disorder diagnosis. Results indicated that across studies, individuals with a diagnosis of panic disorder (PD), social anxiety disorder (SAD), post-traumatic stress disorder, generalised anxiety disorder (GAD), or a specific phobia displayed significantly lower time-domain and/or high frequency HRV than healthy controls. This suggests individuals with anxiety disorders experience a lower level of parasympathetic outflow, which, according to the polyvagal theory and neurovisceral integration model, indicates a reduced ability to control maladaptive autonomic responses (Porges, 1995; Thayer & Lane, 2000). Interestingly, there was no significant difference in low frequency HRV between anxious and non-anxious participants, possibly highlighting the specificity of the influence of anxiety on the PNS (Chalmers et al., 2014).

Another systematic review and meta-analysis by Shahrestani et al. (2015) examined HRV among adults and adolescents with either clinical or sub-clinical psychopathology as compared to

healthy controls, both at rest and during social interactions. Social interactions included the Trier Social Stress Test, as well as positive, neutral, and negative dyadic interaction tasks. Results showed that participants tended to exhibit an overall reduction in HRV during negative social interaction or social stress tasks when compared to baseline, but did not experience a change in HRV during neutral or positive social interactions (Shahrestani et al., 2015). An additional key finding to emerge from this meta-analysis was that changes in HRV during certain social interactions (e.g. TSST, negative dyadic interaction tasks) may provide a useful index for psychopathology in these groups, and that future studies could usefully examine such patterns in other diagnostic groups (Shahrestani et al., 2015). Sharestani et al. also highlight that respiratory sinus arrhythmia and high frequency HRV seem sensitive to indexing HRV change during negative social interaction, and highlight the need to examine other indices of HRV.

Finally, a meta-analysis and review by Kim et al. (2018) synthesised studies examining HRV at rest and during stress tasks in healthy participants. A number of different stress tasks were examined, such as simulation of a medical emergency, periods before a university examination, state and trait anxiety questionnaires, and mental tasks such as the stroop task. While results were inconsistent, the most frequently reported variation in HRV due to stress tasks was a decrease in parasympathetic activity, where high frequency HRV decreased, and low frequency HRV increased (Kimet al., 2018). They also report findings that suggest increases in stressful situations were associated with an increase in sympathetic nervous system activity (as indexed by an increase in the LF/HF ratio, where low frequency HRV increases and high frequency HRV decreases), and a decrease in RMSSD. It is suggested that the vagus nerve may serve as a 'structural link' between the brain and the heart, one that is indexed by HRV. Thus, it is proposed that many indices of HRV are associated with threat perception, and may index overall autonomic flexibility and health (Thayer et al., 2012; Kim et al., 2018). Kim et al. state that while HRV could be a useful objective measure of stress and mental health, consistent biological measures can be hard to acquire in individuals with psychopathology, as there are many factors that influence psychiatric disorders, besides physiology alone. Thus, when examining HRV in clinical settings, or in the context of acute stressors, it is important to take into account all other factors, including the medical and psychological history of an individual (Kim et al., 2018).

In sum, the evidence to date suggests there is a clear relationship between HRV and psychopathology, at least during rest. Chalmers et al. (2014) found that individuals with an anxiety disorder displayed significantly lower time-domain and high frequency HRV during recordings at rest. Shahrestani et al. (2015) found that acute social stress or negative social interactions also had the capacity to reduce HRV, even in healthy controls. Kim et al. (2018) also suggest that there is likely a

correlation between HRV and stressful situations in healthy controls. A number of individual studies have examined HRV levels of those with an anxiety disorder compared to controls both at baseline and under conditions of elevated state anxiety during a disorder-relevant stress task. However, to date, there has been no systematic review or meta-analysis specifically examining the pattern of effects on HRV measures between these groups during disorder-relevant stress. Examining the correlation between HRV and stress will have a number of important implications. Firstly, it will help to examine whether common physiological theories of anxiety (e.g. the polyvagal and neurovisceral integration models), which approach anxiety in a more trait-like manner, still apply during disorder relevant stress. This will allow us a more holistic view of physiological responding in anxiety disorders, where we can account for physiology in a wider group of scenarios. Furthermore, examining physiology during stress will have clinical implications, as it will allow us to determine the most efficient ways to diagnose and treat anxiety using physiology. For example, if stress tasks do not further differentiate anxious and control participants compared to at baseline, we can conclude that it is unnecessary to subject participants to stress in order to diagnose anxiety disorders using physiological responding. The present meta-analysis aims to expand on the work of Chalmers et al. and Shahrestani et al. by examining whether HRV better differentiates between individuals with versus without an anxiety disorder at rest or during disorder specific stress (state anxiety). This meta-analysis will focus specifically on DSM-5 (APA, 2013) anxiety disorders including PD, SAD, GAD, and specific phobias. This meta-analysis will address questions regarding the pattern of HRV observed between clinically anxious and controls under rest, under stress, and the degree of change between them. Consistent with past findings, the first hypothesis is that the meta-analysis will verify the finding that clinically anxious participants will display significantly lower levels of HRV than controls groups at rest. The second question considered concerns the degree of change in HRV observed between rest and stress across groups. Based on past evidence, two outcomes appear possible. Firstly, it is hypothesised that the difference observed at baseline is simply maintained under stress. Alternatively, it is possible that anxious participants will display an even greater decrease in HRV during stress. The third question considers the influence of stress tasks themselves on HRV. It is expected that control participants will display lower levels of HRV during stress, compared to baseline. Similarly, it is expected that anxious individuals will display lower levels of HRV during stress, compared to baseline.

Methods

Search Criteria

Peer reviewed studies were identified using Scopus, PsycINFO, Web of Science, Proquest, Medline, and Embase, and pooled together using EndNote. Databases were searched using all relevant combinations of the following phrases: “HRV,” “heart rate variability,” “state anxiety,” “stress,” “anxi*,” “fear,” “fearful,” “social anxiety,” “social anxiety disorder,” “social phob*,” “social anx*,” “SAD,” “fear of negative evaluation,” “fear of positive evaluation,” “(fear adj3 evaluati*),” “social interaction,” “social performance,” “generali!ed anxiety,” “GAD,” “worry,” “ruminat*,” “panic disorder,” “panic*,” “agoraphobia,” “agoraphob*,” “specific phobia,” and “phob*”

Inclusion and exclusion criteria

Studies with the following features were included: (a) Quantitative, (b) written in English, (C) peer-reviewed, (d) original, (e) full text available, (f), compared a group of individuals with a DSM-5 anxiety disorder to healthy controls both during baseline and during exposure to a disorder-relevant stressor, and (g) clearly measured HRV during both baseline and the disorder-relevant stressor. Studies using earlier versions of the DSM to diagnose disorders were included, but only if the anxiety disorder is also classified as a DSM-5 anxiety disorder. Studies with the following features were excluded: (a) animal studies, (b) participants under 18 years of age, (c) did not use a structured clinical interview to confirm diagnosis, (d) reviews, and (e) qualitative study. No timeframe was set on the search. Studies that included participants who were taking psychotropic medication or had comorbidities were included. These participant characteristics were extracted as potential moderator variables.

Figure 1 illustrates the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart. Following removal of irrelevant articles based on a title review, abstracts were reviewed and more irrelevant articles were removed. Full texts of the remaining articles were removed, which yielded nine articles meeting the inclusion criteria. Characteristics for the final sample of studies can be seen in Table 1. Most full texts examined were excluded due to participants having no clinical diagnosis (*n* =12), no HRV measurement (*n* = 8), or no disorder-relevant stress tasks (*n* = 7). Nine authors were contacted for means and standard deviations for key measures, however none were able to provide these statistics. Seven additional studies were identified through the search of included studies’ references, however none met all inclusion criteria. Two other independent screeners examined a total of 20 percent of abstracts collectively for inter-rater reliability. The overall concordance rate for abstracts between viewers was 97.4%, with

disagreements resolved through discussion. A full protocol for this study was pre-registered using PROSPERO (https://www.crd.york.ac.uk/prospero/ CRD42021240558)

Figure 1

PRISMA flowchart Illustrating Article Selection

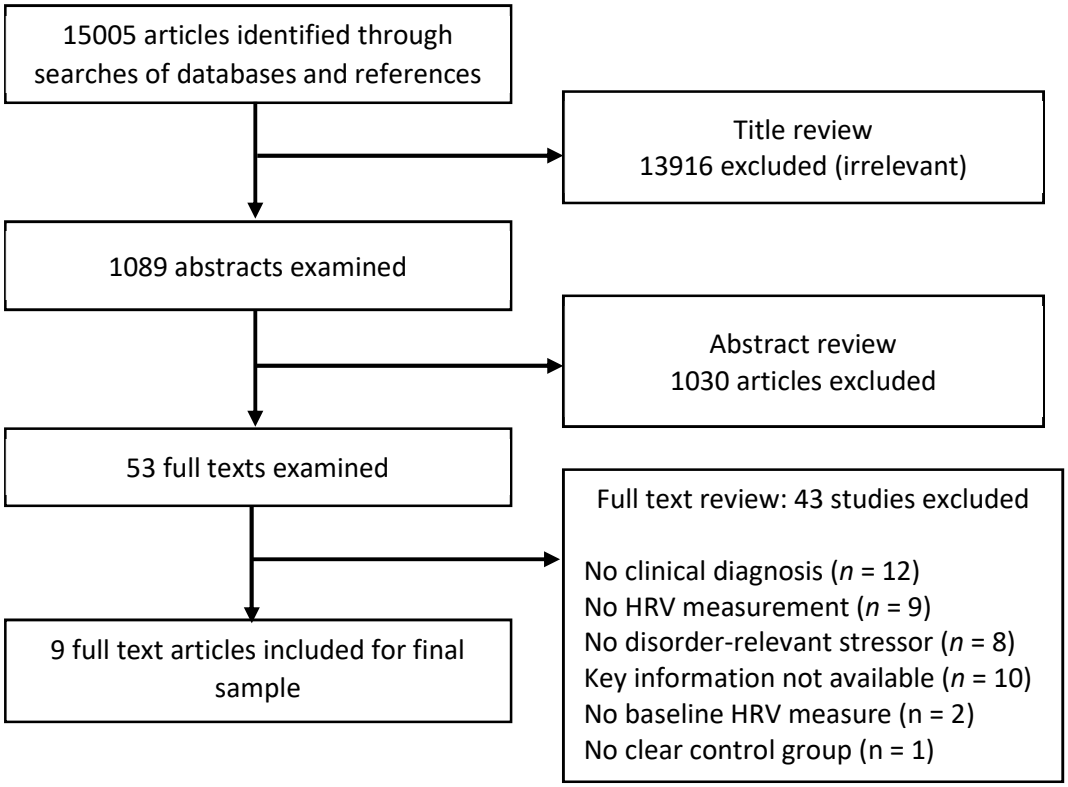


Table 1

Characteristics of Included Studies

Study	HRV measures	Anxiety Disorder (*n*)	Healthy controls (*n*)	Disorder examined	Stress task used
Cohen, et al. (2000)	MSD, HF, LF	11	25	Panic disorder	Recall of panic inducing memory
Garakani, et al. (2008)	MSD, HF, LF	43	11	Panic disorder	30 breaths/min x 10 mins
Martinez, et al. (2015)	HF, LF	15	30	Panic disorder	Doxapram
Seier, et al. (1997)	HF, LF	10	11	Panic disorder	Lactate
Breuninger, et al. 2017)	HF	21	27	Panic disorder + Agoraphobia	Virtual reality stress
Bornas, et al. (2006)	RMSSD, HF, LF	61	58	Flight phobia	Threatening flying sequence
Lyonsfield, et al. (1995)	MSD	15	15	Generalised anxiety disorder	Worry induction
Hammel, et al. (2010)	MSD, HF, LF	16	19	Generalised anxiety disorder	Worry induction
Levine, et al. (2016)	HF	10	10	Generalised anxiety disorder	Worry induction, with imagery

Note. MSD = Mean of successive RR differences. HF = High frequency. LF = Low frequency. RMSSD = root mean square of successive RR differences.

Primary outcome measures

The studies identified through search in this study reported both time-domain and frequency-domain measures of HRV. Only two studies included RSA, so the index was not extracted for this review. The time-domain measures extracted included the mean of successive RR differences (MSD), as well as the root mean square of successive RR differences (RMSSD), while the power-domain measures included high frequency HRV and low frequency HRV. Similar to Chalmers et al.'s (2014) study, one time-domain index was calculated across studies and RMSSD was given preference over other time-domain indices where possible, as it most closely represents parasympathetic

activity. Both high frequency and low frequency HRV were examined independently for frequency-domain measures.

Data synthesis and analysis

Means and standard deviations were pooled from all studies and transformed into Hedges' *g*, which has the advantage of accounting for biases introduced through smaller sample sizes (Cujipers, 2016). Data were analysed using JASP (JASP team, 2019). The meta-analyses were conducted using a random effects model to account for random error and variation in effect sizes between different studies (Cujipers, 2016). Effect sizes were interpreted as small, medium, and large at 0.2, 0.5, and 0.8 respectively (Cohen, 1998).

Heterogeneity of effect sizes between studies was examined using the Q-test. A significant result suggests there is evidence for heterogeneity, meaning that some amount of variance between studies can be accounted for due to methodological or sample differences (Cujipers, 2016). Along with this measure, the I^2 statistic was used to quantify the level of heterogeneity. The I^2 statistic represents the percentage of total variance that can be accounted for due to heterogeneity, and can range from 0% to 100% (Cujipers, 2016).

Between-group comparisons were conducted separately at baseline and then under stress. Within-group comparisons were then conducted separately in samples with and then without an anxiety disorder diagnosis, to determine whether HRV indices changed when under stress. Analyses were conducted separately for three HRV indices; high frequency, time-domain, and low frequency HRV. Publication bias was examined using Egger's regression. A significant *p* value for the test indicates publication bias may be present (Lin & Chu, 2018). Similar to Chalmers et al.'s (2014) meta-analysis, we considered moderator analyses for confounds such as medication use and psychiatric comorbidities, however, due to the small number of studies available, moderator analyses were not possible.

Results

This analysis included 202 clinical participants and 206 control participants. Disorders analysed included panic disorder, agoraphobia, specific phobias, and generalised anxiety disorder. One SAD study met criteria, but could not be contacted for data necessary to meta-analyse (Klumbies et al., 2014; See study 2).

Does HRV in clinical and control groups differ at rest?

The comparison of individuals with versus without an anxiety disorder fell just short of statistical significance on baseline high frequency HRV and the effect size was small to moderate (Hedges' g = -0.38, p = .051). The Q statistic was significant and there was high heterogeneity

between studies (Q = 16.34, p = .012, I^2=67.28). Time-domain HRV was significantly lower in individuals with versus without an anxiety disorder during baseline and the effect size was large (Hedges' g = -1.00, p = .001), suggesting that individuals with an anxiety disorder experienced lower levels of time-domain HRV while at rest than those without an anxiety disorder. The Q statistic was significant and there was high heterogeneity between studies (Q = 27.89, p<.001, I^2 = 84.20). There was no significant difference in low frequency HRV between individuals with versus without an anxiety disorder and the effect size was small (Hedges' g = 0.17, p = .440), although there was significant and high heterogeneity between studies. (Q = 14.25, p = .014, I2=68.16). Egger's regression was not significant for any analysis, indicating no concern for publication bias (See table 2).

Does HRV in clinical and control groups differ under stress?

Individuals with versus without an anxiety disorder did not significantly differ on high frequency HRV when under stress and the effect size was small (Hedges' g = -0.11, p = .345). The Q statistics was not significant and heterogeneity between studies was small (Q = 4.61, p = .595, I^2 = 3.74). Time-domain HRV was significantly lower in individuals with versus without an anxiety disorder and the effect was large (Hedges' g = -0.74, p = .003), suggesting that individuals with an anxiety disorder experience lower levels of time-domain HRV under stress than individuals without an anxiety disorder. The Q statistic was significant and heterogeneity between studies was high (Q = 15.95, p = .003, I^2=72.61). There was no significant difference in low frequency HRV between individuals with versus without an anxiety disorder and the effect size was small (Hedges' g = 0.08, p = .499. The Q statistic was not significant and there was low heterogeneity between studies (Q = 5.57, p = .350, I^2=2.87). Egger's regression was not significant for any analysis, indicating no concern for publication bias (See Table 2).

Do controls show change in HRV from baseline to stress?

We then examined the within-group differences (baseline vs. stressor) for the non-clinical control samples. High frequency HRV did not significantly change from baseline to stressor and the effect size was moderate (Hedges' g = -0.50, p = .221). The Q statistic was significant and there was high heterogeneity between studies (Q = 25.16, p <.001, I^2 = 90.27). Time-domain HRV also did not significantly change from baseline to stressor and the effect size was moderate to large (Hedges' g = -0.682, p = .107. The Q statistic was significant and heterogeneity was high (Q = 95.86, p <.001, I^2 = 92.95). Finally, low frequency HRV did not significantly change from baseline to stressor and the effect size was moderate (Hedges' g = 0.50, p <.001). The Q statistic was significant and heterogeneity was high (Q = 33.88, p <.001, I^2 = 91.12). Egger's regression was not statistically

significant for high frequency HRV or time-domain HRV, but was significant for low frequency HRV, indicating possible publication bias (see Table 2).

Do clinically anxious individuals show change in HRV from baseline to stress?

Finally, we examined the within-group differences for the clinical samples. High frequency HRV did not significantly change from baseline to stressor and the effect size was moderate (Hedges' g = -0.50, p =.221). The Q statistic was significant and there was high heterogeneity between studies (Q = 25.16, p <.001, I^2 = 90.27). Time-domain HRV also did not significantly differ from baseline to stressor and the effect size was moderate to large (Hedges g = -0.68, p = .107). The Q statistic was significant and there was high heterogeneity between studies (Q = 95.86, p <.001, I^2 = 92.95). Low frequency HRV also did not significantly change from baseline to stressor and the effect size was moderate to large (Hedges' g = .50, p = .244). The Q statistic was significant and heterogeneity between studies was high (Q = 33.88, p <.001, I^2 = 91.12). Egger's regression was not significant for any analysis, indicating no concern for publication bias (See Table 2).

Table 2

HRV Results from Meta Analyses

Meta-analysis performed	Group/Time differences			Cochran's Q		I^2	Egger's regression	
	ES (CI)	SE	*p* value	Q statistic	*p* value		Z statistic	*p* value
Effect of disorder (during baseline)								
HF	-0.38 (-0.76 to 0.00)	0.19	.051	16.34	.012	67.28	0.99	.320
TD	-1.00(-1.60 to -0.40)	0.31	.001	27.89	<.001	84.20	-0.34	.733
LF	0.17 (-0.26 to 0.60)	0.29	.440	14.25	.014	68.16	0.74	.457
Effect of disorder (during stress)								
HF	-0.11 (-0.32 to 0.11)	0.11	.345	4.61	.595	3.74	-1.57	.117
TD	-0.74 (-1.23 to -0.26)	0.25	.003	5.57	.350	72.61	0.90	.367
LF	0.08 (-0.15 to 0.31)	0.12	.499	15.95	.003	2.87	-0.97	.333
Effect of stressor (clinical group)								
HF	-0.62 (-1.25 to 0.01)	0.32	.052	84.44	<.001	90.55	0.57	.577
TD	-0.52 (-1.32 to 0.23	0.41	.200	58.64	<.001	92.51	0.17	.862
LF	0.37 (-0.46 to 1.20)	0.42	.385	25.92	<.001	90.94	2.42	.016
Effect of stressor (control group)								
HF	-0.50 (-1.31 to 0.30)	0.41	.221	25.16	<.001	90.27	-1.48	.139
TD	-0.68 (-1.51 to 0.15)	0.42	.107	95.86	<.001	92.95	1.16	.246
LF	.503 (-0.34 to 1.35)	0.43	.244	33.88	<.001	91.12	.409	.683

Note. HF = high frequency (*n controls* = 185, *n anxious* = 177). TD = time domain (*n controls* = 258, *n anxious* = 146). LF = low frequency (*n controls*= 158, *n anxious* = 161). ES = Effect size. CI = Confidence intervals. SE = Standard error.

Discussion

This study aimed to examine whether HRV better differentiated between individuals with and without an anxiety disorder at rest or during disorder specific stress. Previous reviews have examined HRV in anxious and non-anxious individuals at rest (Chalmers et al. 2014), but none have compared HRV in these groups both at rest and during a disorder-relevant stressor. Hypothesis 1 was supported, as participants with an anxiety disorder displayed lower levels of HRV at baseline. Hypothesis 2 was also supported, as this difference remained during disorder-relevant stress. Hypothesis 3 and 4 were not supported, as the introduction of a stress tasks did not significantly influence physiological responding, either in the anxious or control groups.

Individuals with an anxiety disorder typically displayed significantly lower levels of HRV than individuals without an anxiety disorder, both at rest and in the context of a disorder-relevant stressor. This pattern of findings indicates that during baseline, individuals with an anxiety disorder display lower levels of parasympathetic nervous system activity, as indexed by time-domain and high frequency HRV (with a large and small-to-moderate effect size, respectively). This pattern is consistent with theoretical models such as the polyvagal theory, which posit that individuals with an anxiety disorder are less able to regulate ANS arousal, and thus experience lower levels of PNS activation (Porges, 2003; Thayer & Lane, 2000). This is also consistent with the findings from Chalmers et al.'s (2014) meta-analysis, which found lower levels of time-domain and high frequency HRV in anxious participants at rest, compared to healthy controls. Our study extended Chalmers et al.'s findings to include a disorder-relevant stressor.

Time-domain HRV was also significantly lower in anxious participants than controls during disorder-relevant stress tasks. Again, this result was in line with the polyvagal theory, as this pattern of findings indicates anxious individuals were less able to regulate their arousal during stress (Porges, 2003; Thayer & Lane, 2000). Low frequency HRV did not differ between anxious and control participants at rest, suggesting no difference in sympathetic nervous system activity, which reflects ANS arousal. This is consistent with Chalmers et al.'s (2014) review, which found no difference in low frequency HRV between anxious and control participants at baseline. There was also no difference in low frequency HRV between anxious and control participants during disorder-relevant stressors, and the effect size was small, indicating that ANS arousal did not differ between groups in these assessment points either.

Interestingly, results showed no evidence that disorder-relevant stress tasks reliably influence HRV, when compared to baseline (rest). HRV measures did not significantly change from rest to stress tasks in either the control group or the anxious group, indicating that participants' overall ANS functioning did not show consistent change under stress, when compared to baseline.

However, given that the effect sizes related to these non-significant findings were observed to be moderate, it is possible that this non-significance may be attributable to insufficient statistical power. The pattern of effects indicates that parasympathetic activity and ANS regulation (as indexed by high frequency HRV and RMSSD) has decreased in both groups from baseline to stressor, while sympathetic activity and ANS arousal has decreased (as indexed by low frequency HRV). Should these effects be replicated and found to be significant in a sufficiently powered study, this would suggest that disorder relevant stress tasks have decreases autonomic regulation and increased arousal in both the control and clinical groups. However, no firm conclusions can be drawn from this study.

The between-group findings from this review have potentially important implications for how we view the physiological response in anxiety disorders. While high frequency and/or time-domain HRV were lower in anxious participants during the rest and stress conditions, there was no statistically significant difference in low frequency HRV between anxious and control participants during either assessment point. This would suggest that anxious and non-anxious participants experience similar levels of ANS arousal (as indexed by low frequency HRV), but the degree to which they are able to regulate that arousal differs (as indexed by high frequency and time-domain HRV). This remains the case not only at baseline (while resting), but also while engaged in anxiety-inducing scenarios.

The within-group findings from this review suggest that anxiety-inducing scenarios themselves do not further differentiate physiological responding between individuals with versus without anxiety disorders with respect to PNS functioning. The stressor tasks examined in this review did not significantly affect any indices of HRV in either the control or anxious groups. This indicates that neither group experienced higher levels of physiological arousal (as indexed by low frequency HRV), or subsequent regulation of that arousal (as indexed by high frequency and time-domain HRV) during anxiety inducing scenarios when compared to baseline. These results alongside the differences found between groups, could indicate that trait levels of PNS functioning (as indexed by high frequency and time-domain HRV) rather than acute responses to stressful stimuli (e.g. by seeing a reduction in HRV from baseline to stress) predicts anxiety diagnosis.

These results are consistent with theoretical physiological models of anxiety such as the polyvagal theory, which places an emphasis specifically on an imbalance between sympathetic and parasympathetic outflow, where sympathetic arousal is too high, and parasympathetic regulation is too low (Porges, 2003). The theory states that for survival, it is necessary for mammals to differentiate between safe and unsafe environments. When a safe environment has been identified, vagal outflow increases, allowing regulation of visceral states associated with sympathetic arousal,

promoting calm states and social behaviours (Porges, 2003). It is hypothesised that anxiety disorders are associated with an inability to identify safe environments, causing a prolonged state of vagal withdraw, reducing an individual's ability to regulate maladaptive autonomic responses (Porges, 2003). This is consistent with the findings of the present meta-analysis, as individuals with anxiety appear to be less able to regulate their arousal (as indexed by high frequency HRV and RMSSD), regardless of safe (baseline) or potentially threatening (disorder-relevant stressor) environments. It may be the case that individuals with an anxiety disorder are not able to effectively identify the perceived threats (or lack of) in these environments, leading to a trait level reduction in ANS regulation.

Similarly, the generalized unsafety theory of stress (GUTS; Brosschot et al., 2018) hypothesises that the physiological stress response is constantly 'on' and is only inhibited by the prefrontal cortex when an individual deems their environment as safe. Long-term stress responses are thus a result of an individual erroneously perceiving unsafety, rather than a result of stressors themselves (Brosschot et al., 2018). From a physiological perspective, this is consistent with results from this review, where anxious individuals experience the same level of physiological arousal, but are less able to inhibit arousal than healthy controls. This further supports the idea that anxious individuals have a fundamental problem differentiating between safe and unsafe environments.

The findings from this study have a number of implications for the diagnosis and treatment of anxiety disorders. It seems that HRV effectively differentiates between anxious and non-anxious individuals at baseline just as well as during disorder-relevant stress. Thus, if using physiological responding specifically as a biomarker for anxiety, the present evidence suggests that it would be unnecessary to subject individuals to acute stress in order to elicit such anxiety-linked physiological indicators. It also seems that physiological regulation (as indexed e.g. by high frequency HRV and RMSSD) acts as a better predictor of anxiety than physiological arousal (as indexed e.g. by low frequency HRV).

Our results suggest that it would make little sense to target physiological arousal itself directly in the aims of reducing symptom severity of anxiety, as sympathetic arousal is not observed to be consistently higher in anxious individuals. Rather, if aiming to complement cognitive behavioural approaches to treatment with a focus on physiology, the present results suggest that a focus on regulatory processes would be most appropriate. Potential examples of how this could be achieved can be seen in Gross' (2015) process model of emotion regulation, where he describes two general classes of emotion regulation strategies; antecedent-focused regulation and response-focused regulation. One commonly explored technique within antecedent-focused regulation is that of cognitive reappraisal, where an individual pre-emptively modifies their cognitive appraisal of a

situation in order to change their emotional reaction to the situation (Gross, 2015). Response modulation, a response-focused technique, is where an individual aims to influence their behavioural or physiological response to an emotion after that emotion has been triggered (Gross, 2015). Cognitive reappraisal is often viewed as an adaptive long-term strategy, as it is associated with decreased levels of negative emotional experience and sympathetic response, whereas response modulation can lead to decreased positive but not negative emotional experience, and an increase in sympathetic arousal (Gross, 2015). Thus the model predicts that how an individual views a situation plays a key role in regulation of emotional and physiological response, which is consistent with the polyvagal theory.

Similar to Gross' (2015) process model, the polyvagal (Porges, 2003) and GUTS (Brosschot et al., 2018) theories highlight that an individual's inability to identify safe environments is a key contributing factor to unhealthy physiological and emotional processes associated with many anxiety disorders. When considering the role physiology has in the maintenance of anxiety, future treatment avenues may place an emphasis on an individual's cognitions surrounding their environment and their physiological symptoms. Gross' process model specifically places an emphasis on antecedent-focused emotional regulation strategies, which is supported here by the trait-level reduction in ANS regulation individuals with anxiety experience. Inhibitory learning-based approaches to treating anxiety also emphasise the importance of reappraising physiological symptoms and contexts and forming new non-fear associations, rather than unlearning fear associations or habituating the sympathetic arousal (Craske et al., 2014).

This study aimed to synthesise relevant HRV data from all DSM-5 (APA, 2013) anxiety disorders. Unfortunately, very few studies on specific phobias and agoraphobia, and no studies on social anxiety met the inclusion criteria. This limits the inferences we can make about HRV in these disorders. Furthermore, the ANS may be influenced by a myriad of factors other than anxiety, such as other mental disorders, age, sex, or medication use, and HRV is only part of the overall picture when examining regulatory processes in the face of perceived threats or stress. Due to the limited number of studies available, we were unable to perform any moderator or follow-up analyses for this review to explain the high heterogeneity across samples. Thus, building up a greater pool of studies specifically examining HRV in anxiety disorders at rest and during disorder-relevant stress would allow more detailed analyses, leading to a more precise understanding of anxiety. Future studies examining agoraphobia, specific phobias, or social anxiety disorder are in the most need, given the lack of current literature surrounding these disorders.

This review examined whether HRV at rest or during disorder-relevant stress better differentiates between individuals with versus without an anxiety disorder. Results found that

anxious individuals are characterised by trait-like lower levels of parasympathetic activity (as indexed by high frequency and time-domain HRV), but that stress tasks did not influence HRV in either the anxious or non-anxious group. These results are consistent with common physiological models of anxiety, such as the polyvagal theory and GUTS (Porges, 2003; Brosschot et al., 2018). Future studies could usefully build on the present findings by examining HRV under rest and stress conditions for under-researched disorders such as SAD, and by further examining the interactive influence of cognitive processes such as reappraisal on physiological responding in anxiety disorders. This would allow a more holistic understanding of how HRV interacts with cognitions, and whether acute stress itself has any influence on these interactions.

Chapter 3

Abstract

Cognitive behavioural models of social anxiety disorder (SAD) typically deemphasise the role of physiological factors that may influence treatment response and maintenance of the disorder. Examining underlying physiological processes associated with SAD can provide a more complete understanding of the disorder. Previous studies have shown that heart rate variability (HRV), an index of autonomic nervous system functioning, is associated with SAD when measured at rest. However, there is little research examining whether HRV at rest or during social stress better differentiates between individuals with SAD and healthy controls. Participants (*N=153*) were treatment-seeking individuals with a principal diagnosis of SAD (*n = 94;* M_{age} = 28.97), or healthy controls (*n = 59;* M_{age} = 21.56) over 18 years. Physiological data were collected from participants both at rest and during a standardised social interaction and performance task (i.e. the Trier Social Stress Test). HRV was compared at baseline, as well as during the social stress tasks. Results revealed that HRV increased from baseline to stress in both groups, but that HRV did not differentiate between socially anxious participants and controls, either at rest or during social stress. Results indicated that while psychological stress influences HRV, no evidence was found in support of the prediction that objective physiological arousal differentiates between individuals with and without a SAD diagnosis.

How Does HRV Differentiate Between Individuals With Versus Without SAD at Rest and During Social Stress

Social anxiety disorder (SAD) is characterised by an intense fear of social situations, whereby individuals believe they will be scrutinised and negatively evaluated by others (American Psychiatric Association [APA], 2013). For an individual with SAD, social situations almost always provoke fear or anxiety that is disproportionate to the actual threat posed by social situations (APA, 2013). SAD can have a debilitating effect on everyday functioning, with clinical populations commonly experiencing impairment in areas such as their social life, work or education (Aderka et al., 2012). Once a relatively neglected condition, SAD is identified now as one of the most prevalent psychiatric disorders around the world (Stein et al., 2017). Historically, SAD has predominantly been examined through models that emphasise cognitive behavioural aspects of the disorder (e.g., Wong & Rapee, 2016). More recently, however, there has been a growing interest in other modalities, such as physiological influences, that may underlie maintenance of SAD and inform future treatment avenues (Hyett et al., 2018). The aim of this study was to examine the relationship between SAD and physiological parameters of autonomic arousal.

One study examined the HRV levels of participants with SAD compared to healthy controls at rest (while no social stress is taking place; Alvares et al., 2013). Participants completed a number of self-report measures, as well as a measure of HRV for a 5-minute rest period. It was found that participants with SAD reported statistically significantly lower HRV than participants without SAD. Lower levels of HRV were also associated with higher levels of symptom severity, fear, and avoidance.

While Alvares et al. (2013) showed a clear relationship between HRV and symptom severity in SAD, psychophysiological indices were acquired while participants were at rest. Given that HRV may help to explain underlying physiological processes associated with SAD occurring during social behaviour, it would be beneficial to examine the effect of HRV on SAD during social stress, rather than just at rest (NIMH, 2016; Porges, 1995). For example, the generalised unsafety theory of stress posits that individuals with an anxiety disorder cannot differentiate between safe and unsafe environments, and thus are always physiologically aroused as they are anticipating a threat (Brosschot et al., 2018). By examining SAD both at rest and during social stress, we can see how physiological responding changes, and if there are indeed changes, in a ‘safe’ vs. ‘unsafe’ environment. Furthermore, in their generic cognitive model, Beck and Haigh (2014) detail the importance of maladaptive adaption to stimuli within everyday life as a maintaining factor of many disorders. When our adaptive systems over-react to life stressors, we experience psychological

distress, leading even to diagnosable disorders (Beck & Haigh, 2014). By examining physiology during disorder relevant stress tasks, we can deduct whether our physiological adaptive systems (such as the PNS and SNS branch) are also reacting in a maladaptive fashion. An effective way of simulating social environments in a laboratory-based setting is the Trier Social Stress Test (TSST; Kirschbaum et al., 1993). The TSST includes both social performance and social interaction tasks that aim to elicit mild psychological distress (Kirschbaum et al., 1993). The social performance task involves the delivery of an unstructured speech, where participants are to convince an audience that they are the best candidate for a hypothetical job opening of their choosing. The social interaction task involves a general conversation between participant and researcher. A number of independent studies have shown that the TSST reliably induces a number of indices of stress, including hormones such as cortisol (Kirscbaum et al., 1993), but few studies have investigated HRV during social stress.

One study used a modified version of the TSST to compare indices of HRV before and after single-session group treatments (Hyett et al., 2018). This study compared three groups; two groups diagnosed with SAD who received different versions of a CBT intervention (verbally-based CBT & imagery-enhanced CBT), which were compared to a waitlist control group. The verbally-based CBT used verbal-linguistic techniques, with no specific reference to mental imagery, while the imagery-enhanced CBT encouraged patients to multi-sensory negative imagery related to social situations (McEvoy et al., 2017). As hypothesised, emotional regulation, indexed by HRV, showed a greater increase during social stress following imagery-enhanced therapy in comparison to the other groups (Hyett et al., 2018). This finding suggests a greater physiological flexibility following the 'enhanced' treatment compared to the alternate treatment and waitlist control (Hyett et al., 2018; Quintana et al., 2016). In contrast, there were no significant differences between the groups in heart rate or skin conductance, suggesting that all groups found the social stressor tasks arousing, but that the imagery-enhanced group regulated their arousal more adaptively. Interestingly, self-report measures of anxious arousal did not differentially change across the groups. This finding may suggest that HRV during the social stressor tasks acted as an early sign of change, one that has not yet been detected through self-report measures.

Klumbies et al.'s (2014) study is another that utilised the TSST to examine physiology in social anxiety compared to controls. Physiology was measured through a number of indices, including HRV (RMSSD - a predominantly parasympathetically influenced index). Results revealed that socially anxious individuals did not differ on HRV compared to healthy controls at baseline or during the social stress task (Klumbies et al., 2014), indicating no group difference in parasympathetic activity. While SAD participants showed no difference in physiological stress parameters, they did report higher levels of subjective stress. It may therefore be the case that

individuals with SAD have a higher sensitivity to anxiety symptoms rather than differential physiological responding per se (Klumbies et al., 2014).

At present, research into the influence of anxiety on HRV is still limited and results are mixed. Alvares et al. (2013) found that SAD is associated with lower levels of HRV at rest, however, Klumbies et al. (2014) found no difference in PNS activation between individuals with SAD and controls either at rest or during social stress. These studies are the first of their kind to examine HRV in both a SAD group and control group at baseline (and during stress in the case of Klumbies et al., 2014). To the best of our knowledge, no study has examined both PNS and SNS activation indexed by measures of HRV in socially anxious and control participants, both at rest and during social stress.

To increase our understanding of psychophysiological factors associated with SAD, and further strengthen the evidence for what we do already know, it is important to compare both branches of the ANS in socially anxious and control participants, both at rest and during social stress. This allows us to examine both a measure of parasympathetic and sympathetic activity through indices of HRV, allowing us to gain a better understanding of the intricacies of the relationship between HRV and ANS functioning. In this study differences in HRV will be compared between a clinical SAD population and healthy controls at baseline, as well as during a social stressor task (TSST). The first hypothesis (trait hypothesis, consistent with the findings from study 1) is that individuals with SAD will show consistently lower levels of HRV, regardless of context (baseline vs. social stress). An alternative hypothesis is that individuals with SAD will show lower levels of HRV during baseline, but will show similar physiological activation to controls under social stress. This finding would suggest that people with SAD are more strongly differentiated from people without SAD by their trait rather than state level of HRV (trait-not state hypothesis). Another possibility is that there will be an even greater difference of HRV levels between groups during social stress compared to baseline, further differentiating these groups (trait-state potentiation hypothesis).

Methods

Research Design

This study employed a 2x2 mixed between-within factorial design. The between-groups independent variable was group (SAD vs. controls). The within-groups independent variable was assessment point (baseline vs stress). The effects of the independent variables on three dependent variables of HRV was examined (high frequency HRV, low frequency HRV, & RMSSD). This study is part of a larger project that had already collected data with the clinical sample (McEvoy et al., 2017; 2020).

Participants

Pre-treatment data from a clinical sample (M_{age} = 28.97, SD = 1.22). who participated in a randomised controlled trial of CBT for social anxiety disorder (McEvoy et al., 2017, 2020) were compared to data collected from a non-clinical sample (M_{age} = 21.56, SD = .45) using the same procedures. Non-clinical participants were Curtin University undergraduate students over 18 years old. To achieve a sample that did not comprise those with clinical or high levels of social anxiety, the study advertisement explicitly stated that researchers were looking to recruit participants who do not get overly anxious in social situations such as conversing with peers, or meeting new people. Inclusion in the final analysis was also dependent on screening for mental health problems (using the Structured Clinical Interview for DSM-5, as described below), with those reporting a history of SAD being excluded from the final control sample (although still permitted to participate for pedagogical reasons). Demographics for the sample can be seen in Table 3. A G*Power a priori analysis was conducted to determine adequate sample size. Assuming a small effect (f=0.15), a power of 80% and an alpha of 0.05, a sample size of 90 was required. We have over-recruited to account for data acquisition errors, as well as unknown effect sizes in an under-researched area.

Table 3

Participant Characteristics

Sample characteristics	Controls *n* (%)	Clinical *n* (%)
Sex		
Male	21 (36)	49 (52)
Female	38 (64)	45 (48)
Education Level		
Less than Year 12	1 (2)	11 (12)
Year 12	47 (80)	38 (40)
Technical/Trade	7 (12)	23 (25)
Tertiary	4 (7)	22 (23)
Employment Status		
Employed	45 (76)	52 (45)
Unemployed	14 (24)	42 (55)
Additional Disorders		
Major Depressive Disorder	18 (31)	38 (40)
Generalised Anxiety Disorder	6 (1)	37 (39)

Note. n controls = 59. *n* clinical participants = 94.

Measures and Apparatus

Structured Clinical Interview for DSM-5

For the control sample the structured clinical interview for DSM-5 (SCID-5; First et al., 2016) screener was administered for social anxiety, major depressive disorder, and generalised anxiety disorder, with the full modules being administered if screens were positive. The SAD module was administered to ensure control participants did not meet diagnostic criteria, as this was an exclusion criterion. The generalised anxiety and major depressive disorder modules were administered for descriptive purposes. For the clinical sample, the full SCID-5 was administered (see McEvoy et al., 2020).

Social Interaction Anxiety Scale & Social Phobia Scale

The Social Interaction Anxiety Scale (SIAS) is a self-report measure of anxiety related to social interaction, while its companion measure, the Social Phobia Scale (SPS) is a self-report measure that assesses an individual's fear of being scrutinised while being observed. (Mattick & Clarke, 1998). Both measures consist of 20 items answered on a Likert-type scale ranging from 0 ("Not at all characteristic of me") to 4 ("Extremely characteristic of me"). The SIAS includes items such as "I am tense mixing in a group." The SPS asks about both physical symptoms (e.g. "I worry about shaking or trembling when I'm watched by other people") and cognitive symptoms (e.g. "I become self-conscious when using public toilets")**.** The SPS and SIAS discriminate well between socially anxious and non-anxious samples, and correlate well with established measures of social anxiety. They have high levels of discriminant validity and internal consistency (Mattick & Clarke, 1998). For this sample specifically, Cronbach's alpha was .808 for the SIAS and .916 for the SPS, which represent good and excellent levels of internal consistency, respectively (George & Mallery, 2003).

Trier Social Stress Test

The Trier Social Stress Test (TSST) aims to induce moderate levels of psychological stress in a laboratory setting. (Kirschbaum et al., 1993). HRV was first measured at baseline for 5 minutes, preceding any social stress tasks. Participants were then instructed to prepare for their speech for 3 minutes, after which they delivered a speech for 3 minutes. Participants were audio and video recorded for the speech, where they were asked to imagine they were undergoing an interview in which they must convince the audience (the researcher in this case) that they are the best candidate for a hypothetical job they have applied for. This was followed by a 5-minute period in which to engage in an unstructured conversation. Participants were advised to treat the social interaction as a

normal conversation, as you would with a friend or colleague. A number of standardised questions were used by the researcher throughout the interaction, such as “what do you think your family and friends especially like about you?” A number of independent studies have shown that the test induces an increase in heart rate as well as a number of stress hormones (Kirschbaum et al., 1993). The speech task specifically was used as the stress task for this study as it induced the greatest differences in HRV from baseline in the clinical sample (see McEvoy et al., 2020) and in previous research (Hyett et al., 2018).

Demographics & medication

Demographic questions such as age, sex and medication were also administered. This includes questions such as “What medication did you take during the last 3 months?”

Measuring equipment and physiological data

A video camera was placed in the laboratory to record audio and video of each participant’s speech. HRV was collected via 3-lead electrocardiography using a BioPac MP150 data acquisition and analysis system running AcqKnowledge software. Heart beat data were screened for outliers and artefacts using MATLAB (Higham et al., 2016). Artefacts were determined through visual inspection. Of the original 216 files, 34 were removed due to excessive artefacts (15.74%) and 98 were corrected for minor artefacts (45.37%). High frequency HRV, low frequency HRV, and RMSSD was then calculated from raw data using Artiifact, an electrocardiogram processing software (Kaufmann et al., 2011).

Procedure

The clinical sample utilised in this study was originally collected for use in a randomised control trial of CBT for social anxiety (McEvoy et al., 2020). Initially, 107 participants with SAD were recruited from a specialist mental health service that provides psychological treatments for individuals with emotional disorders. Participants were assessed for inclusion criteria by a clinical psychologist. These included; age of 18 years or greater, DSM-5 (APA, 2013) SAD diagnosis, stable medications for the previous month, and willingness to be randomised to two different CBT techniques (McEvoy et al., 2017). Exclusion criteria included; a pre-existing diagnosis of bipolar disorder and/or psychosis, receiving current treatment for SAD, and a high risk of suicide or self harm. Following the separate recruitment for clinical and control participants, the study procedures for the lab testing session were consistent across all participants.

Upon arrival, participants were given an information sheet and consent form, followed by an opportunity to ask any questions. Participants then completed all self-report measures, which included the SIAS and SPS (additional measures were included in the larger study; see McEvoy et al., 2017). Two electrodes were placed under each collarbone and one on the inside of the left hipbone

of participants to measure HRV, and a further two were placed on the non-dominant hand to measure skin conductivity (not assessed in the current study). Participants were then seated in front of the video camera. The researcher then left the room for 5 minutes during which HRV at baseline was measured. Participants were instructed to relax with eyes open for the duration of the baseline period, and not to use mobile phones. Following recording of baseline measures, the participant moved on to the stress section of the TSST. HRV was recorded throughout each phase of the TSST. The speech task was used as the stress condition for this experiment. Participants were seated for the duration of the experiment.

Data Analysis

Assumption testing for the ANOVAs included normality testing using the Shapiro-Wilk test for normality and visual inspection of histograms, Homogeneity using Levene's test, as well as an inspection for outliers and missing data. Little's MCAR test on the physiological responding revealed data to be missing completely at random (p = .479; Li, 2013), thus, expectation maximisation was used to handle missing data. A total of 5.2% of HRV data from the overall sample was missing on the speech task, while no data was missing during baseline. Data were examined using a 2x2 mixed model ANOVAs, with the between-groups factor being group (SAD vs. non-SAD) and the within-groups factor being assessment point (baseline vs. during social stress). All analyses were re-run controlling for age and sex but this did not change the pattern or significance of any findings, so only the uncontrolled results are reported. Due to administrative loss of data, information for the medication use of the non-clinical group was unavailable. To explore the impact of medication use with the available data (medication use for the clinical sample), a 2x2 mixed model ANOVA was run using only the clinical sample, and medication use being employed as the between-groups factor. Results found no significant effects at either assessment point. Effect sizes for the ANOVAs are indexed by eta-squared. Each index of HRV was analysed separately (RMSSD, high frequency HRV, and low frequency HRV).

Results

Twelve cases were removed due to missing data on all physiological measures. A total of 153 participants were included in the final analyses. Descriptive statistics for each sample on each measure can be seen in Table 4. Normality and homogeneity of variance was examined using the Shapiro-Wilk statistic and a visual inspection of histograms and Q-Q plots, as well as Box's test of equality, $F_{max,}$ and Levene's test for equality of variances. All assumptions were met, except for the Shapiro-wilk test for normality. For any positively skewed variables, data were log-transformed and re-analysed, however this had no effect on the significance or direction of any effects. Outliers were

initially screened using standard deviations. Seven cases were identified with scores exceeding ± 3.3 *SD*, suggesting the presence of univariate outliers (Tabachnick & Fidell, 2013). All cases were then examined using Cook's distance. No cases were identified with a Cook's distance value greater than 1, including any case with a z score greater than 3.3, suggesting no univariate outliers were influential. Multivariate outliers were examined by comparing Mahalanobis' distance to a chi-square distribution with the same degrees of freedom. Seven cases were identified as exceeding the Chi-square distribution, and thus, identified as multivariate outliers. These cases were removed. Descriptive statistics for each sample on each measure can be seen in Table 4.

Independent samples t-tests were examined to determine whether groups differed on SIAS and SPS scores. As expected, the clinical group scored significantly higher on both the SPS and SIAS (*M* = 46.30, SD = 16.14 *M* = 57.70, SD = 9.21**,** respectively) than the control group (*M* = 28.75, SD = 8.10**,** *M* = 38.00, SD = 12.60, respectively), with *p*s <.001 for both tests. *d* = 1.85, *d* = 1.31 for the SIAS and SPS respectively. Age between the clinical and non-clinical group differed significantly, t(35.65) = 4.68, *p* <.001, *d* = .77. A chi-square test examining sex differences between the groups was statistically significant, χ^2 (df = 1) = 3.99, *p* = .046, indicating a statistically significant difference in sex. Bivariate correlations for all variables can be seen in Table 5.

Table 4

Descriptive Statistics for HRV Indices for Controls and the SAD Groups at Baseline and Stress

	Baseline		Stress	
Measures	Control M (SD)	SAD M (SD)	Control M (SD)	SAD M (SD)
HF HRV	430.36 (348.26)	460.77 (371.59)	717.22 (406.71)	645.77 (439.97)
RMSSD	32.11 (11.34)	34.32 (13.91)	46.96 (14.47)	46.20 (15.68)
LF HRV	339.63 (276.77)	435.85 (432.73)	812.58 (516.05)	808.80 (515.47)

Note. SAD = social anxiety disorder, HF = high frequency. RMSSD = root mean square of successive differences in heartbeat. LF = low frequency. Control group *n* = 59. SAD group *n* = 94.

Table 5

Bivariate Correlations for HRV Indices

Variable	1	2	3	4	5	6	7	8
1. RMSSD baseline	-							
2. RMSSD speech	.67**	-						
3. RMSSD social	.63**	.78**	-					
4. HF baseline	.87**	.60**	.54**	-				
5. HF speech	.66**	.86**	.66**	.59**	-			
6. HF social	.60**	.66**	.87**	.59**	.65**	-		
7. LF baseline	.35**	.34**	.36**	.29**	.43**	.39**	-	
8. LF speech	.39**	.61**	.47**	.35**	.66**	.53**	.37**	-
9. LF social	.32**	.50**	.59**	.25**	.53**	.69**	.46**	.65**

Note. N = 153. HF = high frequency. RMSSD = root mean square of successive differences in heartbeats. LF = low frequency. *p = ≤ .05 ** p <.01

Analysis 1 - High Frequency HRV

Results can be seen in Table 6 and Figure 2. The main effect of assessment point was statistically significant, indicating that high frequency HRV increased during social stress compared to baseline. In contrast, the main effect of group was not statistically significant, providing no evidence of a group difference of high frequency HRV between socially anxious individuals and controls. The assessment point by group interaction was also not significant.

Analysis 2 - RMSSD

The main effect of assessment point was statistically significant, indicating that RMSSD increased from baseline to social stress. In contrast, the main effect of group was not significant, providing no evidence for a difference on this measure between socially anxious individuals and controls. The assessment point by group interaction was also not significant.

Analysis 3 - Low Frequency HRV

The main effect of assessment point was statistically significant, whereby low frequency HRV was higher during the speech task, when compared to baseline. In contrast, the main effect of group was not significant, indicating that low frequency HRV did not significantly differ between socially anxious individuals and controls. The assessment point by group interaction was also not significant.

Table 6

HRV Results From Mixed Model ANOVAs

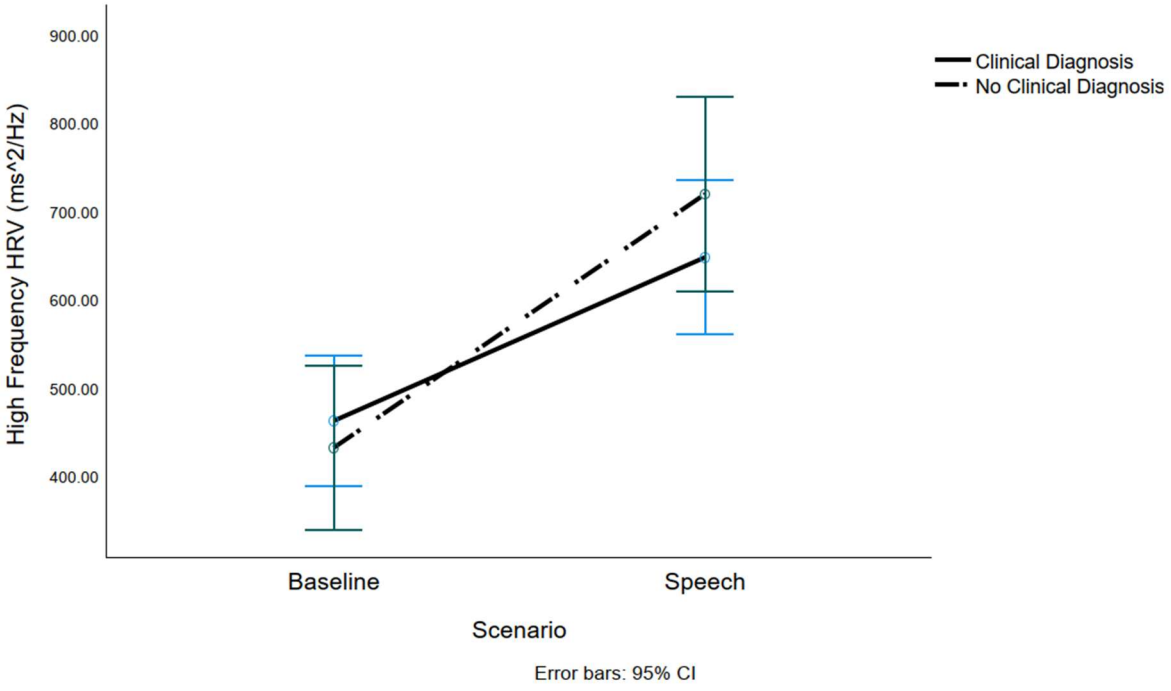
HRV index	Effect of assessment point (stress)			Effect of group (disorder)			Interaction effect		
	F	*p*	n^2	F	*p*	n^2	F	*p*	n^2
HF HRV	61.50	<.001	.289	0.12	.727	<.001	2.87	.093	.019
RMSSD	194.50	<.001	.563	0.12	.735	.001	2.39	.124	.016
LF HRV	98.16	<.001	.394	0.56	.457	.004	1.37	.243	.009

Note. HF = High frequency. RMSSD = Root mean square of successive differences in heartbeats. LF = low frequency

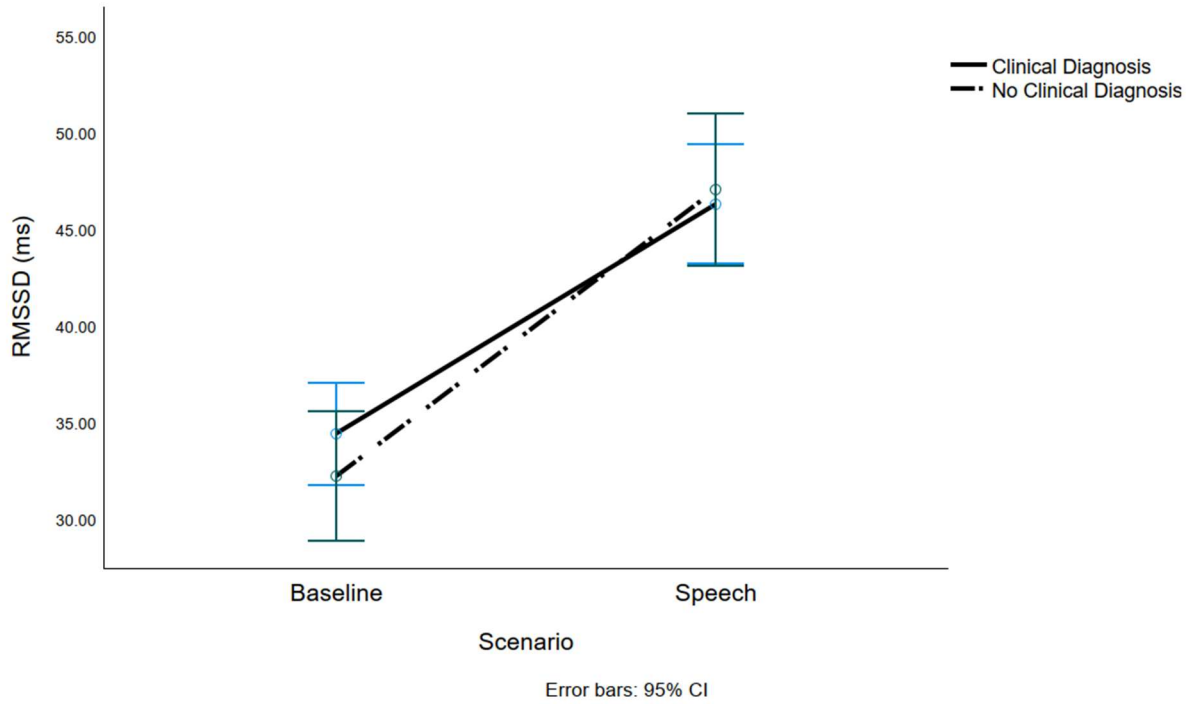
Figure 2

Physiological Response of SAD and Control Participants during Baseline and Speech Phases of the TSST

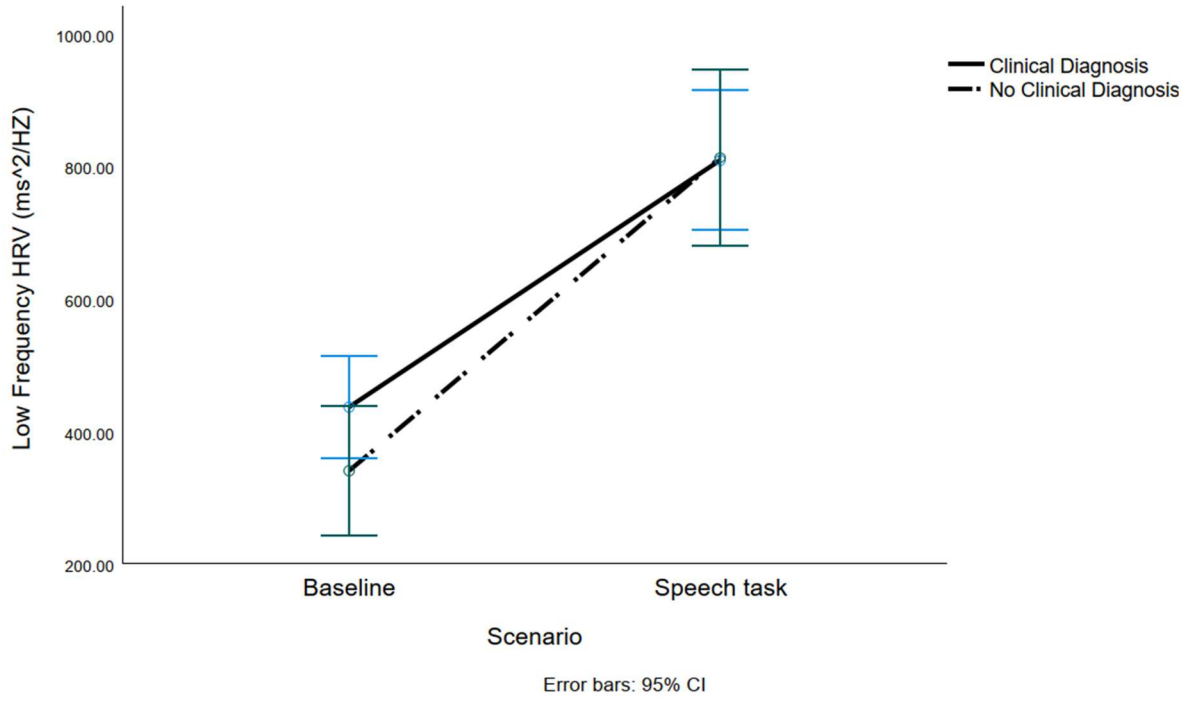
a) HF HRV



b) RMSSD



c) LF HRV



Note. HF = High frequency. RMSSD = root mean square of successive RR differences. LF = Low frequency. HRV = Heart rate variability.

Discussion

This study aimed to determine whether HRV better differentiated socially anxious and non-socially anxious individuals at rest or during social stress. Previous studies have examined HRV as a predictor of SAD during rest, but very little research has explored this relationship during stress.

Klumbies et al.'s (2014) was the only study to date to have examined HRV in both groups at baseline and during social stress. The present study built upon this research by also examining high frequency and low frequency HRV in anxious and non-anxious individuals. Firstly, it was hypothesised that those with SAD would show consistently lower levels of HRV regardless of assessment point (baseline vs. social stress; trait hypothesis). This trait hypothesis was not supported, as there were no between-group differences in HRV identified either at baseline or during social stress. The study also sought to differentiate potential alternative hypotheses concerning the impact of stress on HRV among socially anxious and control participants. Specifically, these hypotheses were that those with SAD would show lower levels of HRV during baseline, but would experience similar levels of physiological responding as controls during social stress (trait-not state hypothesis), or that there would be an even greater difference from baseline to stress in physiology between groups (trait-state potentiation hypothesis). These alternative hypotheses were also unsupported.

High frequency HRV and RMSSD did not differentiate between anxious and non-anxious individuals, either at rest or during social stress. As such there was no evidence that overall parasympathetic activity (as indexed by high frequency HRV and RMSSD) differed between the SAD and control group. Thus, SAD participants were no less able to regulate SNS activity than the control group, regardless of TSST phase. Low frequency HRV also did not differentiate between the two groups, either at rest or during social stress highlighting a concurrent absence of elevated sympathetic arousal (as indexed by high frequency HRV) for the SAD group relative to controls, regardless of TSST phase.

The results for the between-group differences in this study appear inconsistent with common physiological theories of anxiety such as the polyvagal theory and neurovisceral integration model, which hypothesise that reduced ability to control maladaptive autonomic processes (indexed here by high frequency HRV and RMSSD) is a key component of many anxiety disorders, and thus, anxious individuals typically display lower levels of HRV (Porges, 2003; Thayer & Lane, 2000). The results are also inconsistent with Alvares et al.'s (2013) study, which found that socially anxious individuals displayed lower levels of HRV at rest. They are, however, consistent with the only other study investigating HRV in individuals with and without SAD during the TSST (Klumbies et al., 2014), which found no difference in RMSSD between socially anxious and non-socially anxious individuals at rest or during social stress. Extending from Klumbies et al.'s (2014) study, these results also found that low frequency HRV did not differ between groups at either rest or during social stress, suggesting that levels of ANS arousal did not statistically significantly differ between groups at any point of assessment.

Within-group differences were found for both the socially anxious and control groups. Surprisingly, all 3 indices of HRV (high frequency HRV, RMSSD, low frequency HRV) increased from baseline to stress in both groups. An increase in sympathetic activity (as indexed by low frequency HRV) is consistent with an increase in autonomic arousal levels, while an increase in parasympathetic activity (as indexed by high frequency HRV and RMSSD) is consistent with an increase in autonomic regulation.

The observed pattern of effects in the current study showed no differences between groups in HRV scores, either at rest or under social stress. One explanation for these findings is that there may genuinely be no difference in physiological responding between individuals with and without SAD, with other cognitive-affective processes determining the subjective severity and impact of social anxiety. This would, however, be inconsistent with common physiological theories of anxiety such as the polyvagal theory (Porges, 2003). Another possibility is that the nature of the TSST task in particular means that between-group differences that potentially exist on such measures were obscured. For instance, if speech tasks are experienced as universally anxiety-provoking, both groups may experience similar and heightened ANS activation when both anticipating (at baseline) and performing the social stressor task. This explanation would explain the discordance between studies finding no difference in HRV between groups while anticipating a speech task (our study and Klumbies et al., 2014), and those that do find a difference when no speech task was anticipated (Alvares et al., 2013). The latter findings might reflect trait differences that cannot be detected between high and low socially anxious groups when perceived social stress during the TSST is anticipated either before or during exposure to a social stressor. It may be that alternative social stress tasks, which are more likely to be experienced benign for individuals without SAD, would be more sensitive to differences in physiological responding between people with versus without SAD. Such alternative stress tasks may include eating or drinking in front of others or simply informal interaction within a social context.

The interplay between physiological responding and cognition is complex, and not yet well understood. Socially anxious participants scored significantly higher on both the SIAS and SPS, self-report measures of social anxiety symptom severity, than healthy controls. It may be that cognitive factors such as attentional focus and beliefs about physical sensations (e.g. Clark & Wells, 1995; Rapee & Heimberg, 1997) mediate subjective self-reported anxiety and distress more than objective levels of physiological responding. As described in Rapee and Heimberg's (1997; see also Heimberg et al., 2010) cognitive behavioural model of SAD, individuals with SAD form a mental representation of how they are perceived by others, informed by a number of sources, including physiological arousal. They tend to be hyper-vigilant of arousal, fearing it will be seen by others, and they will be

evaluated or scrutinised because of it (Rapee & Heimberg, 1997). In the current study, it is possible therefore, that while the SAD group experience similar objective levels of arousal as the control group, they may be more sensitive to such physiological arousal which in turn serves to maintain social anxiety.

Surprisingly, results revealed that all 3 indices of HRV increased from baseline to stress, for both the socially anxious and control participants. Specifically, indices of both autonomic arousal (low frequency HRV) and regulation (high frequency HRV, RMSSD) increased from baseline to stress in both groups. Specifically, it is surprising that indices of regulation have increased under stress. The results, however, are consistent with Diamond et al.'s (2017) study, which examined autonomic responses to a diagnostic interview. The study found that individuals with GAD experienced a coactivation in PNS and SNS activity from baseline to diagnostic interview. As in the current study, this situation combines a potential stressor that requires the participant to concurrently access higher level cognitive processes to clearly articulate themselves. It may be the case that in such situations involving heightened stress and cognitive performance, PNS activity, or regulation, increases in an attempt to regulate the higher levels of SNS arousal experienced during stress to reduce the impacts of SNS arousal and facilitate performance.

Consistent with this perspective, as well as the correlation between cardiac autonomic activity and emotional control, Thayer and Lane (2000) also emphasise the role of executive functioning on autonomic activity and vagally determined high frequency HRV. Executive functioning is a broad construct, encompassing a number of higher-order cognitive functions, such as reasoning and planning, problem solving, regulation, and successful execution of behaviours (Williams, P. G., 2010). The higher-order cognitions needed to plan and successfully execute a speech would fall under the broad definition of executive functioning. Thus, it may be the case that alongside the influence of emotional control and arousal, the cognitions required to successfully plan and conduct the speech task are influencing HRV, accounting for the increase in HRV from baseline to stress task, in both groups. It may also be the case for the speech specifically, that movement and speaking itself are influencing HRV. Heart rate naturally accelerates during inspiration and slows during expiration, with vagal outflow being inhibited and restored during inhalation and exhalation respectively (Shaffer & Ginsberg, 2017). It is likely that speaking during the speech task is influencing breathing rate, and thus may partly explain the increase in HRV from baseline to speech.

If the present pattern of results represents an accurate reflection of the genuine pattern of HRV more broadly, this would suggest that HRV may not act as a biomarker of SAD, and that physiological responding is not an effective way to differentiate between individuals with and without SAD, either at baseline or during social stress. While physiological arousal itself did not differ

between groups (but has still increased from baseline to stress), self-report symptom severity as measured by the SIAS and SPS did differ, possibly indicating that socially anxious individuals are more sensitive to their physiological anxiety symptoms than controls. It may be the case that self-reported symptom severity of social anxiety may be more sensitive to subjective cognitive and affective experiences and impacts, which are dissociated from the objective level of physiological activation. If this were the case, it would complement common cognitive behavioural models of SAD (e.g. Clark & Wells, 1995; Rapee & Heimberg, 1997) in the sense that it may be more effective to focus treatment on an individual's cognitions and attitudes surrounding their physiological responding in the face of perceived threats or stress, rather than the physiological responding itself. Socially anxious individuals erroneously judge how others will perceive them based on internal cues of arousal levels (Clark & Wells, 1995). Through cognitive restructuring, therapists can challenge beliefs surrounding arousal such as "everyone will judge me because I'm shaking so much", by considering the belief logically and providing disconfirming evidence for it (Beck et al., 2005). The results from this study may be further evidence 'against' these beliefs, as it shows that psychophysiological responding during social stress itself may not be enough to differentiate between individuals with and without SAD. This evidence can be used to complement video-feedback, which is also an effective approach for challenging mental representations of the self, including how obvious physical symptoms are to observers **(**Harve et al., 2000; Rapee & Hayman, 1996). Our findings suggest that the way someone feels about their symptom severity (as indexed by the SIAS and SPS here) is unrelated to their objective levels of arousal. Thus, it may make it easier for socially anxious individuals to believe that their arousal in social situations does not need to be a concern with respect to their social performance. This idea could be further examined by measuring state-level anxiety symptom severity (i.e. while stress is taking place) alongside HRV and trait-level anxiety symptom severity (i.e. the SIAS and SPS).

Due to the necessity of informed consent, participants in the current study were aware that they would be required to deliver a speech and the conditions under which this would occur. As such it is entirely possible that during the baseline assessment phase where individuals were also anticipating the impending speech task. It may be the case that this anticipation could reduce between-group differences at baseline compared to studies that only include a baseline assessment (e.g. Alvares et al., 2013). Future studies could establish a 'true' baseline, where participants are unaware of an upcoming speech, although this obviously raises ethical issues regarding informed consent and deception. Alternatively, participants could be invited to participate in two separate studies, the first that only assesses baseline responding after which participants are invited to complete a follow-up study that includes the full TSST (though again, ethical issues regarding

coercive consent potentially arise). To the best of our knowledge, Alvares is also the only study examining HRV in socially anxious vs. control participants at rest. These future studies would help to further support Alvares' research, and the influence of anticipation of a stress task on HRV. Future studies could also investigate if different social stress tasks (e.g., social interactions with authority figures, eating in front of others, activities designed to elicit social evaluation in public) yield a similar pattern of findings, or whether between-group differences are evident in social stressors perceived to be milder or more specific to socially anxious participants. These study additions may help to clarify whether there genuinely is no difference in physiological responding between socially anxious and control participants, or if the stressful nature of the speech task and its anticipation is obscuring these differences, thus informing whether targeting physiology in SAD as a treatment avenue would be fruitful. Future studies could also examine cognitions and self-reported anxiety alongside HRV in SAD, to further disentangle the complex relationships between physiological responding, cognitions and affect. Due to an administrative loss of data in the non-clinical sample, information for medication use was lost. Thus, medication could not be controlled for throughout the analyses. Given the likelihood that a non-clinical sample would report much lower levels of medication use, and the non-significant results of analyses examining medication use in the clinical sample (see data analysis), it seems unlikely that accounting for medication use across groups would have altered the pattern of findings in this study. However, should future studies aim to replicate or extend these results, examining medication use in both samples would be beneficial.

This study aimed to extend the literature surrounding HRV and SAD by examining whether HRV better differentiates individuals with and without SAD at rest or during social stress. This study included both indices of PNS (high frequency HRV and RMSSD) and SNS (low frequency HRV) functioning. Results found no between-group differences in HRV, either at rest or during social stress, but that the introduction of a speech task significantly increased all indices of HRV in both groups. It may be the case that anticipation of a speech task is obfuscating the between-group differences in this study. Future studies could aim to establish a 'true' baseline, where HRV can be accessed without anticipating a social stressor. Future studies could also usefully seek to examine cognitions alongside HRV in SAD, helping to disentangle the complex and not yet deeply understood interplay between cognitions, physiology, and subjective anxiety. Furthermore, a study could examine state-level, acute symptom severity of anxiety while a stress is taking place alongside a trait-level measure and HRV, to further examine the interplay between physiological responding and cognitions surrounding that responding. Finally, due to administrative error information on medication was missing for the control group. While we undertook analyses to attempt to control for this error (see data analysis; page 27), future studies could stratify by medication type (e.g. SSRIs,

benzodiazepines) to further explore if these confounders influence between group differences in HRV. Nicotine and caffeine use, as well as BMI could also be examined.

Chapter 4

Overall synthesis and conclusions

Results from the meta-analysis (study 1) found that indices of parasympathetic activity (high frequency and time-domain HRV) were significantly lower in anxious samples compared to controls, both at rest and during disorder-relevant stress tasks, indicating that individuals with an anxiety disorder consistently experienced lower levels of autonomic regulation than those without an anxiety disorder. Indices of sympathetic activity (low frequency HRV), however, did not differ between groups during either rest or during stress, suggesting that levels of autonomic arousal do not differ between groups. No measure of HRV differed from baseline to stress in either the anxious or control groups, suggesting that stress tasks do not influence either autonomic arousal or regulation. Thus, the introduction of stress tasks did not further differentiate anxious and non-anxious individuals.

In contrast to study 1, results from the experimental investigation in study 2 found that no measure of HRV differed between socially anxious and control participants, either at rest or during social stress, indicating that neither autonomic regulation (parasympathetic activity; high frequency HRV and RMSSD) nor arousal (sympathetic activity; low frequency HRV) differentiated socially anxious and healthy individuals. All measures of HRV (high frequency HRV, RMSSD, low frequency HRV) increased from baseline to social stress in both groups, indicating that they experienced increased parasympathetic and sympathetic activity during stress. Thus, the social stress task increased both autonomic arousal and regulation in both socially anxious and healthy individuals.

One potential explanation for the discordance in between-group findings between the two studies is the nature of the stress tasks. Specifically, it is possible that the qualities of the Trier Social Stress Test used in Study 2 potentially acts as a more 'universal' stressor, where even non-anxious participants experience stress and anxiety at the prospect of an upcoming speech, diminishing the between-group differences in socially anxious and control participants seen in other disorders with other tasks. The disorder-relevant stress tasks employed in the meta-analysis, however, while effectively stressing the anxious groups, may not have the same influence on the control participants. For example, non-anxious participants may not be concerned about a hyperventilation or worry induction tasks in the PD and GAD studies respectively, emphasising the between-group differences seen both at baseline when potentially also anticipating the stressor, and during the stress task itself. This idea is supported in the SAD literature, as studies where participants were anticipating a speech (study 2; Klumbies et al., 2014) found no between-group differences in HRV,

whereas studies with no anticipation of a speech task (Alvares et al., 2013) did find a between-group difference. This highlights the possibility that a speech task may represent a highly stressful event for socially anxious and control participants alike, and that anticipating an imminent speech may also influence the baseline recordings of HRV.

Common cognitive behavioural models of SAD posit that the generation and maintenance of anxiety occurs initially when an individual anticipates or takes part in a social situation in which there is a perceived audience (Heimberg et al., 2010). For socially anxious participants, a perceived audience brings with it the threat of negative evaluation or scrutiny in a wide number of social situations, ranging from job interviews and formal speeches, to dates or casual social interactions (Heimberg et al., 2010). The speech used in the experimental study was designed as a social performance task, where participants would be judged in the context of a hypothetical job opening. Thus, the social-evaluative nature of the task may well be experienced as stressful both for the socially anxious and control participants, at baseline during anticipation, and during the speech itself potentially resulting in similar physiological arousal for both groups. Where the groups may differ, however, in relation to the cognitions surrounding this arousal during social performance, as seen in scores on the SPS, a self-report measure of social performance anxiety. Cognitive behavioural models of SAD posit that a key factor of the disorder is an individual's perception of themselves. Their self-image is often erroneous and negative, and can be informed by a number of sources, such as past social experiences, mirrors and photographs of themselves, their behaviour, subtle social cues of others (which are often misjudged as negative) and their own physiological responding (Heimberg et al., 2010). As such, it is possible that although objective arousal levels for both groups may not differ in response to a social-evaluative situation, those with SAD are more sensitive of their arousal, leading to higher self-reported social anxiety symptom severity.

Results from studies 1 and 2 indicate that stress tasks themselves are not necessary to differentiate between anxious and healthy individuals in most anxiety disorders, as HRV at baseline appears to distinguish the groups just as effectively as HRV during stress (except in SAD during the TSST). One implication of this for the use of HRV as a biomarker for treatment outcomes or diagnosis, is that the imposition of a stress on participants may not yield additional relevant information meaning assessment may be adequate at rest. The difference in physiological responding between groups also suggests that directly targeting trait physiology as a treatment option may prove fruitful.

Studies directly targeting physiology in an attempt to reduce symptom severity of stress and anxiety have found promising results. A meta-analysis by Goessl et al. (2017) examined how HRV biofeedback influenced symptoms of anxiety and stress in a number of populations such as those

with high levels of speech and performance anxiety, trait anxiety, or general stress symptoms. HRV biofeedback aims to increase an individual's vagal tone by displaying their real-time HRV measurements (Blum et al., 2019). There are then numerous techniques an individual can use to increase HRV in response to biofeedback (Blum et al., 2019). A common technique is resonance-frequency breathing, where individuals breathe slowly and deeply, in time with the frequency of their HRV data provided, increasing vagally mediated HRV (Blum et al., 2019). Goessl et al.'s meta-analysis found a large and significant within-group effect of HRV biofeedback on anxiety symptoms (Hedges' g = .81), as well as a large and significant between-group effect of HRV biofeedback when compared to other treatments (Hedges' g = .83) such as sham biofeedback, muscle relaxation, or treatment as usual. The most commonly used self-report measure in this analysis was the State-Trait Anxiety Inventory (Goessl et al., 2017). These results indicate that HRV biofeedback can significantly improve self-reported symptom severity of anxiety and stress, indicating that targeting physiological parameters of stress and anxiety, can indeed influence self-report symptom severity, further highlighting the interplay between cognitions and physiological responding.

There are still few studies examining how HRV differs between clinical anxiety samples and controls both at baseline, and during disorder-relevant stress. Only 9 studies were included in the final meta-analyses, so we were unable to perform follow-up or moderator analyses. Furthermore, this experimental study was only the second of its kind to examine HRV in SAD and controls at rest and during social stress. Building up a large pool of studies specifically examining anxiety at rest and during stress (particularly social anxiety studies) will allow more detailed analyses and comparisons, such as moderator analyses of specific anxiety disorders, medication, or age, leading to a more complete understanding of physiological processes underlying anxiety. Specifically, there is little research in this area for specific phobias and agoraphobia. While this experimental study adds to the literature surrounding SAD, the discordance in the pattern of effects for self-report (SIAS and SPS scores) and physiological responding (HRV) between groups, highlights the complex interplay between physiology and cognitions in anxiety disorders. Consistent with recommendations from the RDoC initiative (Kozac & Cuthbert, 2016), future studies could examine HRV alongside cognitions and self-reported anxiety, to further our knowledge of relationships between different units of analysis. Future studies in SAD could also attempt to establish a 'true' baseline, one where there is no anticipation of a stressor, to examine whether the pattern of findings in physiology in SAD more closely resembles those found in other anxiety disorders (as seen in the meta analysis), however this brings up ethical concerns with deception. One solution to this is conducting a multi-part study, one where there is a baseline recording, and another on a separate day for the stress task. Alternatively, a study could examine SAD vs. non-sad participants' HRV using a different social stress task, or two

separate studies where the first study involves baseline assessment (with no anticipated social stressor) and the second optional study involves the full TSST.

Conclusions

This research program aimed to examine how HRV differes between clinically anxious and control participants, both at baseline and during disorder-relevant stress. Results of the meta-analysis (study 1) revealed that individuals with an anxiety disorder displayed significantly lower levels of HRV than controls, however, the introduction of a disorder-relevant stressor did not influence physiological responding in either groups. Results of the experimental study (study 2) found that HRV did not differentiate between individuals with versus without social anxiety disorder, either at rest or during social stress, however, the introduction of a speech task significantly influenced HRV in both groups. This discordance between findings may be due to the nature of the stress tasks, where the speech task of study 2 acted as a more 'universal stressor' that removed between-group differences. That is, it is possible that the speech task elicited a heightened stress response from all participants regardless of clinical status. By comparison, other stress tasks employed by studies examined in the meta-analysis included stressors that may be more disorder-specific such as hyperventilation, which may not stress a control group in anticipation of, or during the task. Future studies are needed to build a greater understanding of the relationship between HRV and anxiety both at rest and during stress, particularly around the interplay between cognition, subjective emotion, and physiology. Future SAD studies could also introduce a 'true' baseline. This project expands the work of previous meta-analytic and experimental studies examining physiology in anxiety, further extending our knowledge of how HRV differentiates clinically anxious and control participants. The project also reveals important gaps in our knowledge, such as a detailed understanding of the complex interplay between cognitions and physiology in anxiety.

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Supplementary materials

Material 1

Social Interaction Anxiety Scale (SIAS)

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Instructions: This questionnaire asks you to think about social situations. For each question, please circle a number to

Social Interaction Anxiety Scale (SIAS)

Instructions: This questionnaire asks you to think about social situations. For each question, please circle a number to indicate the degree to which you feel the statement is characteristic of you. The rating scale is as follows:

0	1	2	3	4
Not at all characteristic of me	Slightly characteristic of me	Moderately characteristic of me	Very characteristic of me	Extremely characteristic of me

1.	I get nervous if I have to speak with someone in authority (eg teacher, boss)	0	1	2	3	4
2.	I have difficulty making eye contact with others.	0	1	2	3	4
3.	I become tense if I have to talk about myself or my feelings.	0	1	2	3	4
4.	I find it difficult mixing comfortably with the people I work with.	0	1	2	3	4
5.	I find it easy to make friends of my own age.	0	1	2	3	4
6.	I tense up if I meet an acquaintance in the street.	0	1	2	3	4
7.	When mixing socially, I'm uncomfortable.	0	1	2	3	4
8.	I feel tense if I am alone with just one person..	0	1	2	3	4
9.	I am at ease meeting people at parties, etc.	0	1	2	3	4
10.	I have difficulty talking with other people.	0	1	2	3	4
11.	I find it easy to think of thing to talk about.	0	1	2	3	4
12.	I worry about expressing myself in case I appear awkward.	0	1	2	3	4
13.	I find it difficult to disagree with another's point of view.	0	1	2	3	4
14.	I have difficulty talking to attractive persons of the opposite sex.	0	1	2	3	4
15.	I find myself worrying that I won't know what to say in social situations.	0	1	2	3	4
16.	I am nervous mixing with people I don't know very well.	0	1	2	3	4
17.	I feel I'll say something embarrassing when talking.	0	1	2	3	4
18.	When mixing in a group, I find myself worrying I will be ignored.	0	1	2	3	4
19.	I am tense mixing in a group.	0	1	2	3	4
20.	I am unsure whether to greet someone I know only slightly.	0	1	2	3	4

Material 2

Social Performance Scale (SPS)

Social Phobia Scale (SPS)

Instructions: For each question, please circle a number to indicate the degree to which you feel the statement is characteristic of you. The rating scale is as follows:

0	1	2	3	4
Not at all characteristic of me	Slightly characteristic of me	Moderately characteristic of me	Very characteristic of me	Extremely characteristic of me

1.	I become anxious if I have to write in front of other people.	0	1	2	3	4
2.	I become self-conscious when using public toilets.	0	1	2	3	4
3.	I can suddenly become aware of my own voice and of others listening to me.	0	1	2	3	4
4.	I get nervous that people are staring at me as I walk down the street.	0	1	2	3	4
5.	I fear I may blush when I am with others.	0	1	2	3	4
6.	I feel self-conscious if I have to enter a room where others are already seated.	0	1	2	3	4
7.	I worry about shaking or trembling when I'm watched by other people.	0	1	2	3	4
8.	I would get tense if I had to sit facing other people on a bus or a train.	0	1	2	3	4
9.	I get panicky that others might see me faint or be sick or ill.	0	1	2	3	4
10.	I would find it difficult to drink something if in a group of people.	0	1	2	3	4
11.	It would make me feel self-conscious to eat in front of a stranger at a restaurant.	0	1	2	3	4
12.	I am worried people will think my behaviour is odd.	0	1	2	3	4
13.	I would get tense if I had to carry a tray across a crowded cafeteria.	0	1	2	3	4
14.	I worry I'll lose control of myself in front of other people.	0	1	2	3	4
15.	I worry I might do something to attract the attention of other people.	0	1	2	3	4
16.	When in an elevator, I am tense if people look at me.	0	1	2	3	4
17.	I can feel conspicuous standing in a line.	0	1	2	3	4
18.	I can get tense when I speak in front of other people.	0	1	2	3	4
19.	I worry my head will shake or nod in front of others.	0	1	2	3	4
20.	I feel awkward and tense if I know people are watching me.	0	1	2	3	4

Material 3

Medication Questionnaire

What medication did you take during the last 3 months?

With medication we mean all drugs that were prescribed and medication that may be bought at the pharmacy. See below for an example.

What is the medication name?	***How much did you take per time?***	***How many times did you take this per day?***	***How many days during the past 3 months did you take the medication?***
Example 1 Furosemide (diuretic)	*Example* 40 mg	*Example* 1 time	*Example* 26 days (2 times a week; 90 days)

Material 4

Trier Social Stress Test (TSST) Protocol

Researcher introduces task:

"For the next part we will ask you to attach a number of recording devices to measure your heart rate and skin moisture levels, before, during and after a speech task. We will measure your heart rate and skin moisture levels for 5 minutes at rest before the task. During the speech task itself you will be required to discuss your personal strengths and weaknesses. Finally, following the speech task I will ask you some more questions."

Heart rate (electrocardiogram) electrode placement:

"These electrodes will be used to record your heart rate before, during, and after the task we are about to perform. Place one on the inner side of your left hip bone, and one under each collar bone." Researcher provides participant with some privacy at this point, then guides participant to attach the electrode leads.

Attach electrocardiogram leads:

Researcher to show participant the leads and highlight that the leads will be attached to the electrodes to allow heart rate information to be sent to the recording equipment. Describe how the clip works. Clip each lead to each electrode (BLACK ← UPPER LEFT COLLAR BONE; WHITE ← UPPER RIGHT COLLAR BONE; RED ← LOWER LEFT HIP). Ask participant to be seated (set-up such that experimenter will be seated between table/equipment and door, with participant on opposite side).

Skin sweat response (electrodermal activity) set-up:

Once participant is seated, researcher places two electrodes on participant's non-dominant hand, and attaches leads. Check physiology recording is accurate before starting experiment. If not, make adjustments to set-up (i.e., check placement of electrodes on participant) and then begin.

Video camera set-up

Once participant is comfortably seated, set-up video camera on tripod and then begin the test.

Social stress test - baseline acquisition (5 mins):

Explain to participant: "We will now measure your heart rate and skin response for 5 minutes while you are at rest. Whilst we are recording I will leave the room. Do you have any questions before we start?" Press start on acquisition file (and record on camera) and record for 5 minutes. Leave the room during this period and return after 5 minutes is up. Press stop on the acquisition file and save. Close file.

Social stress test - preparation phase (3-minute preparation):

Read verbatim to participant: "Your task in this experiment is the following: please imagine that you have applied for a job and have been invited for an interview. In contrast to a real interview, however, you are supposed to give a talk, in which you are to convince me, in three minutes, why you think that you would be the best candidate for this position. Please note that you will be recorded by a camera and a microphone for subsequent voice and behavioural analysis. You should try to leave the best possible impression, and assume the role of the applicant for the duration of the talk as best as you can. Following your talk, you will be given a task, which will be explained to you at that point. You may take some notes now for three minutes, which you must not use during your talk. Do you have any questions?"

Provide participant with notepad and pen. The researcher then leaves while the participant remains in room where they have three minutes to prepare for the talk. The participant is allowed to take notes but must not use these notes during their speech in front of the researcher.

Return to room after 3 minute preparation period (stop physiology recording and set-up interview recording file).

Social stress test - free speech (3-minute speech):

- Re-start physiology recording, and then ask participant to begin speech.
- If the participant talks for ***longer*** than 3 minutes, then interrupt and press stop on recording.
- If participant stops ***before*** 3 minutes, wait for about 20s and then say: "You still have time."
- Only after a pause of more than twenty seconds prior to the end of the three minute period are questions asked (e.g., "*Tell me more about that particular strength?*").

- If participant does not continue, press stop on recording. Save file and exit.

Social stress test - interaction (5 minutes):

For the following it is important to treat it like a natural interaction/conversation.

- What do your family/friends especially appreciate about you?
  - [Researcher's standard contribution to the conversation: "If I were to say one thing that my family likes about me, I'd probably say that I am kind…and probably loyal too."]
- What do you appreciate about your friends?
  - [Researcher's standard contribution to the conversation: "If I were to say one thing that I appreciate about my friends, is that I have one friend in particular who would always be there for me no matter what."] ➢ What do you appreciate about colleagues?
  - [Researcher's standard contribution to the conversation: "I really like sharing ideas about new work projects, but also I see them outside of work sometimes, so some of them are like friends as well."]
- Please complete the following sentence: I am the best at/in…" ○ [Researcher's standard contribution to the conversation: "I find this a hard one to answer, but I think I am quite empathic and I try to understand where other people are coming from."]

- If participant gets stuck on any question, say, for example "Can you tell me more about that …" Continue for up to 5 minutes.
- After 5 minutes, press stop on recording. Save file and exit.

Material 5

Information Sheet

Government of Western Australia
Department of Health

Curtin University

Participant Information Sheet/Consent Form

Centre for Clinical Interventions, North Metropolitan Health Service Mental Health

Title	*Cognitive behaviour therapy for social anxiety*
Principal Investigator	*Associate Professor Peter McEvoy*
Project Sponsor	*National Health and Medical Research Council*
Project Coordinator	*Dr Matthew Hyett*

Part 1 What does my participation involve?

1 Introduction

You are invited to take part in this research project because you have been referred to the Centre for Clinical Interventions (CCI) for the social anxiety programme. The research project is comparing two treatments for social anxiety disorder to test whether one programme produces better outcomes than the other. We at CCI know that both are effective but we do not know if one is more effective than the other.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- Understand what you have read
- Consent to take part in the research project
- Consent to have the tests and treatments that are described
- Consent to the use of your personal and health information as described.

2 What is the purpose of this research?

We aim to see whether individuals with social anxiety disorder (SAD) will have improved outcomes from one of two group cognitive therapy programmes. If one treatment is more effective than another then the most effective one will become standard practice at CCI. Both treatments involve 12, weekly, 2-hour sessions plus three follow-up appointments – two appointments at 1-month and another 6 months following treatment completion. Our project will also evaluate processes involved in bringing about change from the two treatments. Lastly, we will compare the cost-effectiveness of the treatments.

3 What does participation in this research involve?

We are looking at recruiting 96 individuals with SAD to the treatment trial. Written informed consent will be obtained from you prior to involvement. Sometimes we do not know which treatment is best for treating a condition. To find out we need to compare different treatments. We put people into groups and give each group a different treatment. The results are compared to see if one is better. To try to make sure the groups are the same, each participant is put into a group by chance (random). There will hence be a 50/50 chance of being in each group.

A CCI clinician has already screened you for provisional eligibility. We request access to the questionnaires that you complete as part of CCI's standard assessment procedures, but you will need to complete additional assessments (with a trial assessing psychologist) if you agree to take part in the research. Prior to your first treatment session you will be required to complete a structured interview, some study specific questionnaires (regarding your use of health services

and social anxiety), and perform a video-recorded speech task during which we will obtain measures of heart rate variability (HRV) and skin conductance level (SCL):

- Heart rate variability is the variation in the interval between heart beats and is an important determinant of overall physical wellbeing; by placing a cuff on your wrist (like a blood pressure cuff) we are able to measure the activity of the heart and hence HRV.

- The SCL is a measure of physiological arousal, which is monitored by sticking sensors to the tips of your fingers.

You will then take part in 12, 2-hour weekly group therapy sessions (with between 8 and 10 other individuals) with a project treating clinical psychologist. You will be asked to complete self-report questionnaires prior to each treatment session so that we can track your progress. These sessions will be audio-recorded so that we can later evaluate whether the project treating clinician covered all the key components of the treatment as intended.

You will be required to attend three follow-up appointments at CCI. The first is a routine 1-month follow-up appointment with the project treating clinician to monitor your progress. An additional 1-month follow-up will be conducted where you will be asked to complete self-report questionnaires relating to social anxiety and psychological wellbeing, and have an assessment interview. A speech task will be readministered at 1-month follow-up. An assessment interview and self-report questionnaires are also administered 6 months following treatment completion.

The total duration of participation in this project is 6 months (from intake to follow-up), with the whole study running for 3 ½ years between June 2016 and December 2019. Treatment progress will be monitored by a project treating clinician.

There are no additional costs associated with participating in this research project, nor will you be paid.

4 Other relevant information about the research project

There are two types of group therapy to be compared in the trial:

1. Imagery-based group cognitive behavioural therapy
2. Verbally-based group cognitive behavioural therapy

The former uses mental imagery techniques to challenge thoughts and modify negative emotional experiences in those with SAD, whereas verbally-based techniques focus entirely on learning new ways to challenge unhelpful thought processes.

The project will be run from the CCI in Northbridge, Western Australia, and is led by Associate Professor Peter McEvoy who is a senior clinical psychologist at CCI and an Associate Professor at the School of Psychology and Speech Pathology at Curtin University. The study will be run in collaboration with colleagues at the Centre for Clinical Interventions, Curtin University, the University of New South Wales and Macquarie University in Sydney, Australia, Cambridge University in the United Kingdom, and the University of Waterloo in Canada. Funding for the project is provided by the National Health and Medical Research Council of Australia (NHMRC Project Grant ID # APP1104007) and Curtin University.

5 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage. If you do decide to take part, you will be given this Participant Information Sheet and Consent Form to sign and you will be given a copy to keep. Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your clinical care, your relationship with those treating you or your relationship with North Metropolitan Mental Health Services or Curtin University.

6 What are the alternatives to participation?

You do not have to take part in this research project to receive treatment at this site. Other options are available; this includes taking part in the standard group therapy sessions for social anxiety disorder at CCI, which also involve 12, weekly, 2-hour sessions plus a follow-up session and will enable you to work on challenging and modifying unhelpful thought processes. The assessing clinician on the study will discuss these options with you before you decide whether or not to take part in this research project. You can also discuss the options with your local doctor.

7 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from taking part in this research. We do, however, expect that both treatments will be beneficial.

8 What are the possible risks and disadvantages of taking part?

This is a psychological intervention that has no known negative side effects. Given the emotional nature of psychological therapy it is possible that you may at times be upset and distressed. Some people may find parts of the research confronting (for example the video-recorded speech task). If you become upset or distressed as a result of your participation in the research, the treating clinician will be able to arrange for counselling or other appropriate support following your trial appointment. Any counselling or support will be provided by qualified staff who are not members of the research project team. This counselling will be provided free of charge.

The trial clinician will monitor your risk to self and others. Negative events such as suicidal ideation will be assessed during the initial assessment and throughout the trial. If there is a risk of clinical worsening this will be managed as per the Centre for Clinical Intervention's risk management protocol and will be documented and reported.

This research project involves the collection of information about your use of drugs and other sensitive medical information (e.g., diagnoses, family history). WA Health or Curtin University may be required to release such information if required by law.

9 What happens if my situation changes?

If you decide to withdraw, the lead study investigator will make arrangements for your regular health care to continue through CCI or other appropriate service as directed by your referring clinician. Also, on receiving new information, the lead study investigator might consider it to be in your best interests to withdraw you from the research project. If this happens, he/she will explain the reasons and arrange for your regular health care to continue.

10 Can I have other treatments during this research project?

Whilst you are participating in this research project you should inform the study investigators about any changes to medications you take. We request that you do not undergo any other form of cognitive behavioural therapy whilst taking part in this research project.

11 What if I withdraw from this research project?

If you decide to withdraw from the project, please notify a member of the research team before you withdraw. You have the right to discontinue your involvement in the trial at any time. You may wish to complete treatment at CCI but discontinue with the trial. The decision to withdraw from this research project will not impact your ability to receive alternative treatment at CCI. The researchers will ask you your reasons for withdrawing so that this can be recorded.

If you do withdraw your consent during the research project, the senior researcher and relevant study staff will not collect additional personal information from you, although information already collected may be retained (if you consent to this) to ensure that the results of the research project can be measured properly.

12 Could this research project be stopped unexpectedly?

Neither treatment is expected to result in high rates of adverse outcomes. However, should new information become available regarding negative side effects across treatment groups, or in one of the treatment groups in particular, the trial will be suspended and the ethics committees informed.

13 What happens when the research project ends?

Participants will be invited to 'opt-in' to receiving information about the treatment outcomes at the conclusion of the study by providing their contact details on the consent form. Participants will be informed that if their contact details change and therefore the investigators are unable to contact them, they will be able to contact the project coordinator to obtain this information. Results are expected to be available towards the end of 2019/early 2020.

Part 2 How is the research project being conducted?

14 What will happen to information about me?

You will be assigned a unique ID, which is stored on a password-protected database containing your personal details, accessible only to select study staff to maintain confidentiality. Hard copy study materials will be kept in secure filing cabinets at the Centre for Clinical Interventions in Northbridge. Electronic records will be kept at CCI and at the School of Psychology and Speech Pathology at Curtin University. Paper-based files will be stored for 25 years, following which they will be shredded and disposed of securely. Electronic records will be retained in a restricted access data repository at Curtin University for 25 years as per the Western Australian University Sector Disposal Authority policy.

15 Complaints and compensation

If you suffer any injuries or complications as a result of participating in this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

16 Who has reviewed the research project?

Approval to conduct this research has been provided by the Human Research Ethics Committees of the North Metropolitan Mental Health Service Research Ethics and Governance Office (NHMS MH REGO) in accordance with their ethics review and approval procedures. Any person considering participation in this research project, or agreeing to participate, may raise any questions or issues with the researchers at any time. In addition, any person not satisfied with the response of researchers may raise ethics issues or concerns, and may make any complaints about this research project by contacting the NMHS MH REGO Executive Officer on (08) 9347 6502 or NMAHSMHREGO@health.wa.gov.au. All research participants are entitled to retain a copy of any Participant Information Form and/or Participant Consent Form relating to this research project.

17 Further information and who to contact

If you want any further information concerning this project or need to discuss matters relating to your participation, you can contact the project coordinator:

Name	Dr Matthew Hyett
Position	Research Fellow, School of Psychology and Speech Pathology, Curtin University
Telephone	+61 8 9266 1399
Email	Matthew.Hyett@curtin.edu.au

Material 6

Consent form

Government of Western Australia
Department of Health

Curtin University

Participant Consent Form

Title	*Cognitive behaviour therapy for social anxiety*
Principal Investigator	*Associate Professor Peter McEvoy*
Project Sponsor	*National Health and Medical Research Council*
Project Coordinator	*Dr Matthew Hyett*

Declaration by Participant

By signing the consent form I declare that:

- I have read the Participant Information Sheet or someone has read it to me in a language that I understand.
- I understand the purposes, procedures and risks of the research described in the project.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the project without affecting my relationship with the organisations involved.
- I understand that I will be given a signed copy of this document to keep.
- I agree to the treatment sessions being tape recorded, and to being video-recorded during the speech task experiments.
- I agree to remain confidential about the group sessions. This means that you will be asked not to talk about other group members or about the session discussions with people outside the group.
- I agree for my data to be retained in a restricted use data repository for 25 years at Curtin University, per the Western Australian University Sector Disposal Authority policy.

Name of Participant (please print) _____

Signature _____ Date _____

Declaration by Trial Assessing Clinician

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Assessing Clinician _____

Signature _____ Date _____

Please provide your contact details below if you wish to receive information about the findings of the study at its conclusion.

Address: _____

Email: _____

Home Phone: _____ Mobile Phone: _____

Material 7

Withdrawal Form

Government of Western Australia
Department of Health

Curtin University

Form for Withdrawal of Participation

Title	*Cognitive behaviour therapy for social anxiety*
Principal Investigator	*Associate Professor Peter McEvoy*
Project Sponsor	*National Health and Medical Research Council*
Project Coordinator	*Dr Matthew Hyett*

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with the Centre for Clinical Interventions.

Name of Participant (please print) _____

Signature _____ Date _____

I give the research team permission to retain and use data already collected from me as part of this research study (please circle):

Yes / No

Reason for study withdrawal (to be completed by study investigator)

Declaration by Senior Researcher

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Senior Researcher
(please print) _____

Signature _____ Date _____

10 Data Collection, Storage, Maintenance, Security and Archiving

Participants will be assigned a unique ID upon consenting to the study, which will be applied to all data sources throughout the trial for all study documentation (e.g., clinical measures) and recordings (e.g., physiological/video-recordings). This unique ID will be linked to a password-protected main database containing patient names, contact details and demographics, which will only be accessible to the lead CI on the project and study coordinator. Consistent with previous SAD trials, participants will be required to complete a video-recorded speech task at baseline and at the individual 1-month follow-up. All paper-based clinical and demographic measures collected at the CCI will be stored in secure filing cabinets at CCI. All de-identified electronic data will be stored in databases on password-protected servers at CCI and at the School of Psychology and Speech Pathology at Curtin University. Physiological data and video files will be stored on password-protected servers accessible only to members of the study team at Curtin University. Data will be kept for a minimum of 25 years following completion of data collection and analysis phases of the project. De-identified electronic data will be deposited in a restricted access data registry (e.g., Curtin University Library Data Repository), which may be used by academic journals for data verification purposes. Paper-based files will be shredded and disposed of using secure waste facilities. Video-recordings of patients' speech tasks and audio-recordings of

treatment sessions will be erased from secure servers on completion of the study after all blind ratings have been taken.

10.1 Procedures for missing and/or unused data

As a procedure for accommodating missing data GLMM out-performs traditional procedures for missing data replacement, including the last observation carried forward procedure.[21] See above section 9.5 for further detail on missing data and participant attrition.

11 Monitoring and Audit

The trial investigators will permit trial-related monitoring, audits and access to data by external sponsors, HRECs and institutional governance review bodies. Monitoring of the trial will be conducted as required by the above agencies, and on an annual basis through formal ethical review with WA Health. This will consist of the completion of adverse event monitoring forms when required, and the submission of annual progress report forms that details participant information (consent, recruitment commencement dates, proposed number of participants), current reporting requirements (i.e., changes over the past 12 months), progress of the project, and a summary of site specific issues.

12 Quality Control and Quality Assurance

This trial will be conducted in compliance with the protocol, CONSORT guidelines, Good Clinical Practice administrative guidelines and application regulatory requirements. All training provided to project staff (both clinical and non-clinical) will be standardised and facilitated by fully qualified project staff (e.g., certification processes are in place for training clinicians in group therapy protocols, and for training clinical masters students in coding the video recorded speech tasks). Inter-rater reliability will be assessed between the 2 video coders.

13 Ethics

Ethical approval will be obtained through relevant institutional HRECs, namely WA Health (NMHS Mental Health HREC) and Curtin University in accordance with the National Statement of the National Health and Medical Research Council of Australia (NHMRC). Informed consent will be obtained from all participants prior to participation (see Participant Information and Consent Form, Appendix D).

14 Budget, Financing, Indemnity and Insurance

The trial is funded from an NHMRC project grant awarded to CI McEvoy, who is employed by Curtin University's School of Psychology and Speech Pathology and North Metro Mental Health Service's Centre for Clinical Interventions. The grant is funding a research coordinator plus a part-time assessing clinician and a part-timer treating clinician. Curtin University's Office of Research and Development is supplementing these salaries. The clinical responsibility for the patients' care will remain with the treating clinicians at CCI. CCI clinicians will continue to run social anxiety disorder groups as they currently are as part of their clinical roles within the service. Any increase in clinical time required to run additional groups should be covered by the grant-funded clinicians.

Curtin University will provide indemnity insurance to those on Curtin contracts working on the trial (trial assessing and treating clinicians, research coordinator etc). Confidentiality agreements will be signed by Curtin staff working within WA Health.

15 Publication

Findings will be disseminated to clinicians, stakeholders, and the community via publications in top-tier academic journals, conference and professional clinical seminar presentations, a published treatment manual, and via mass media (media releases, radio). Participants will be invited to 'opt-in' to receiving information about the treatment outcomes at the conclusion of the study by providing their contact details on the consent form. Participants will be informed that if their contact details change and therefore the investigators are unable to contact them, they will be able to contact the CI to obtain this information.

16 References

1. American Psychiatric Association AP. *Diagnostic and Statistical Manual of Mental Disorders.* 5th ed. Washington, DC: American Psychiatric Association; 2013.
2. National Institute for Health and Care Excellence (NICE). Social anxiety disorder: recognition, assessment and treatment. 2013; guidance.nice.org.uk/cg159.
3. Acarturk C, Cuijpers P, van Straten A, de Graaf R. Psychological treatment of social anxiety disorder: a meta-analysis. *Psychological medicine.* 2009;39(2):241-254.
4. Holmes EA, Mathews A. Mental imagery in emotion and emotional disorders. *Clinical psychology review.* 2010;30(3):349-362.
5. Heimberg RG, Brozovich FA, Rapee RM. A cognitive-behavioral model of social anxiety disorder. In: Hofmann SG, DiBartolo PM, eds. *Social anxiety: clinical, developmental and social perspectives.* 3rd ed. New York: Elsevier; 2014.

Material x

Ethics Approval

Curtin University

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08-Mar-2021

Name: Peter McEvoy
Department/School: School of Psychology
Email: Peter.Mcevoy@curtin.edu.au

Dear Peter McEvoy

RE: Annual report acknowledgment
Approval number: HRE2020-0159

Thank you for submitting an annual report to the Human Research Ethics Office for the project The impact of social stress on physiology in healthy younger adults.

The Human Research Ethics Office acknowledges the project is ongoing and approval will remain current until 01-Apr-2022.

Special Condition of Approval Extension.

It is the responsibility of the Chief Investigator to ensure that any activity undertaken under this project adheres to the latest available advice from the Government or the University regarding COVID-19.

Any special conditions noted in the original approval letter still apply.

Standard conditions of approval

1. **Research must be conducted according to the approved proposal**
2. **Report in a timely manner anything that might warrant review of ethical approval of the project including:**
   - **proposed changes to the approved proposal or conduct of the study**
   - **unanticipated problems that might affect continued ethical acceptability of the project**
   - **major deviations from the HREC approved protocol procedures and/or regulatory guidelines**
   - **serious adverse events**
3. **Amendments to the proposal must be approved by the Human Research Ethics Office before they are implemented (except where an amendment is undertaken to eliminate an immediate risk to participants)**
4. **An annual progress report must be submitted to the Human Research Ethics Office on or before the anniversary of approval and a completion report submitted on completion of the project**
5. **Personnel working on this project must be adequately qualified by education, training and experience for their role, or supervised**
6. **Personnel must disclose any actual or potential conflicts of interest, including any financial or other interest or affiliation, that bears on this project**
7. **Changes to personnel working on this project must be reported to the Human Research Ethics Office**
8. **Data and primary materials must be retained and stored in accordance with the Western Australian University Sector Disposal Authority (WAUSDA) and the Curtin University Research Data and Primary Materials policy**
9. **Where practicable, results of the research should be made available to the research participants in a timely and clear manner**
10. **Unless prohibited by contractual obligations, results of the research should be disseminated in a manner that will allow public scrutiny; the Human Research Ethics Office must be informed of any constraints on publication**
11. **Ethics approval is dependent upon ongoing compliance of the research with the Australian Code for the Responsible Conduct of Research, the National Statement on Ethical Conduct in Human Research, applicable legal requirements, and with Curtin University policies, procedures and governance requirements**
12. **The Human Research Ethics Office may conduct audits on a portion of approved projects.**