# AN EXAMINATION OF FACTORS INFLUENCING THE PHYSIOLOGICAL AND PSYCHOLOGICAL RESPONSES TO ACUTE PSYCHOLOGICAL STRESS

**by**

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## Abstract

The overarching aim of this thesis was to examine factors associated with physiological and psychological responses to stress. Four laboratory-based studies were completed using multi-disciplinary methodologies to assess physiological and psychological responses to acute psychological stress. Chapter 2 revealed amygdala and hippocampus morphology was negatively associated with stressor-evoked cardiovascular reactivity in healthy participants. Chapter 3 demonstrated that compared to a stress only condition with no exercise, engaging in an acute bout of high intensity exercise attenuated blood pressure responses to subsequent acute stress exposure, but induced negative effects for mood and stress appraisals in healthy participants. Chapter 4 revealed that perceived heart rate change rather than actual heart rate reactivity during acute psychological stress was positively associated with anxiety intensity in healthy participants. Chapter 5 demonstrated that compared to healthy individuals, patients with ulcerative colitis experienced differences in inflammation, vagal activity and perceived control during acute psychological stress. In response to stress, negative psychological responses but not physiological responses were associated with greater ulcerative colitis symptom burden. The current thesis demonstrates novel factors associated with physiological and psychological responses to acute psychological stress and reveals mechanisms which could underlie the relationship between stress and disease symptoms in ulcerative colitis.

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2. **Trotman, G. P**., Veldhuijzen van Zanten, J. J. C. S., Ginty, A. T., & Williams, S. E. (under review). Psychological and physiological responses to acute mental stress following acute high intensity exercise. *Psychology of Sport and Exercise*.
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4. **Trotman, G. P**., Kane, K., Cooney, R., Williams, S. E., & Veldhuijzen van Zanten, J. J. C. S. (in preparation). Ulcerative colitis and stress: Psychophysiological responses to acute psychological stress and their association with ulcerative colitis symptom burden.

**During this period of postgraduate study the following empirical papers were also published:**

1. **Trotman, G. P**., Williams, S. E., Quinton, M. L., & Veldhuijzen Van Zanten, J. J. C. S. (2018). Challenge and threat states: examining cardiovascular, cognitive and affective responses to two distinct laboratory stress tasks. *International Journal of Psychophysiology*, 126, 42-51. doi:10.1016/j.ijpsycho.2018.02.004
2. Quinton, M. L., Veldhuijzen van Zanten, J., **Trotman, G. P**., Cumming, J., & Williams, S. E. (2019). Investigating the protective role of mastery imagery ability in buffering debilitative stress responses. *Frontiers in Psychology*, 10, 1657. doi:10.3389/fpsyg.2019.01657

3. Williams, S. E., Veldhuijzen van Zanten, J. J. C. S., **Trotman, G. P**., Quinton, M. L., & Ginty, A. T. (2017). Challenge and threat imagery manipulates heart rate and anxiety responses to stress. *International Journal of Psychophysiology*, 117, 111-118. doi:10.1016/j.ijpsycho.2017.04.011
4. Williams, S.E., Quinton, M., Veldhuijzen van Zanten, J., Davies, J., Möller, C., **Trotman, G.P**., Ginty, A.T. (under review). The benefits of mastery imagery ability during acute psychological stress.

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2. **Trotman, G. P**., Veldhuijzen van Zanten, J. J. C. S., Ginty, A. T., & Williams, S. E. (2017). The associations between heart rate, perceived heart rate and anxiety during acute psychological stress. [Abstract, Poster] 75th Meeting of the American Psychosomatic Society. *Psychosomatic Medicine*, 79(4), A8-A8.
3. Williams, S. E., Veldhuijzen van Zanten, J. J. C. S., **Trotman, G. P**., Quinton, M. L., & Ginty, A. T. (2017). Challenge and threat imagery manipulates heart rate and anxiety responses to stress. [Abstract, Poster] 75th Meeting of the American Psychosomatic Society. *Psychosomatic Medicine*, 79(4), A121-A122.
4. **Trotman, G. P.** (2018). Exercise: it is the chill pill for stress. Presented at the University of Birmingham postgraduate research poster conference, *University of Birmingham*.
5. **Trotman, G. P**., Veldhuijzen van Zanten, J. J. C. S., Ginty, A. T., & Williams, S. E. (2019). The effects of acute exercise on psychophysiological responses to stress. [Abstract, Poster] 77th Meeting of the American Psychosomatic Society. *Psychosomatic Medicine*, 81(4), A86-A86.

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## List of Abbreviations

| | |
|---|---|
| ANOVA | Analysis of Variance |
| B-H | Benjamini-Hochberg |
| BMI | Body Mass Index |
| Bpm | Beats Per Minute |
| CD | Crohn's Disease |
| CO | Cardiac Output |
| DBP | Diastolic Blood Pressure |
| ECG | Electrocardiography |
| FCAL | Faecal Calprotectin |
| HADS | Hospital Anxiety and Depression Scale |
| HPA | Hypothalamic-Pituitary-Adrenal |
| HR | Heart Rate |
| HRV | Heart Rate Variability |
| IAMS | Immediate Anxiety Measures Scale |
| IBD | Inflammatory Bowel Disease |
| ICG | Impedance Cardiography |
| ICV | Intracranial Volume |
| IL-6 / IL-10/ IL-1β | Interleukin-6 / Interleukin-10 / Interleukin-1β |
| MANOVA | Multivariate Analysis of Variance |
| MAP | Mean Arterial Pressure |
| MRI | Magnetic Resonance Imaging |
| MSIT | Multi-Source Interference Task |
| PANAS | Positive and Negative Affect Scale |
| PASAT | Paced Auditory Serial Addition task |
| PEP | Pre-Ejection Period |
| POMS | Profile of Mood States |
| PSQ | Perceived Stress Questionnaire |
| PSS | Perceived Stress Scale |
| PTSD | Post-Traumatic Stress Disorder |

| | |
|---|---|
| RMSSD | Root Mean Square of Successive Differences |
| SBP | Systolic Blood Pressure |
| SES | Socio-Economic Status |
| SV | Stroke Volume |
| TFE | Turbo-Field Echo |
| TNF-α | Tumor Necrosis Factor-Alpha |
| TPR | Total Peripheral Resistance |
| UC | Ulcerative Colitis |
| $VO_{2max}$ | Maximal Oxygen Uptake |

# CHAPTER 1

GENERAL INTRODUCTION

"S*tress is the nonspecific response of the body to any demand made upon it*"

– Hans Selye (1936)

**Stress, Health and Thesis Overview**

In modern society stress is commonplace and is increasingly being recognised as having a significant impact on health. Indeed, according to the Mental Health Foundation (2018), 74% of individuals in the previous year reported feeling so stressed they were overwhelmed and unable to cope. These results of stress have a significant burden on society with 15.4 million working days lost due to work related stress in 2017/2018 (Health and Safety Executive, 2018). Whilst Hans Selye's definition of stress is limited in its simplicity, his original work has stimulated almost a century of research investigating stress' impact on health. Understanding mechanisms through which stress contributes to negative health outcomes and what factors determine an individual's experience of stress is of importance for public health.

In response to a situation which involves novelty, unpredictability and a threat to physical or mental well-being, an individual typically experiences a range of psychological and physiological alterations, which is referred to as psychological stress (Lovallo, 2015). Psychological stress is typically categorised as either acute, whereby an episode typically lasts under 60-minutes e.g., giving a presentation at work, or chronic, whereby the stress is prolonged in nature e.g., continued pressure at work to meet ongoing targets (Benschop & Schedlowski, 1999). During acute psychological stress, an individual interprets perceived threatening situations, evaluates the perceived relevance, and potential risk of harm, which results in a complex interaction of psychological alterations and physiological responses

(Lazarus & Folkman, 1984; Lovallo, 2005; Lovallo & Gerin, 2003; Phillips et al., 2015). From an evolutionary perspective, stress responses are seen as necessary and adaptive, directing attention to danger, stimulating coping behaviours and providing metabolic support to enable efficient fight or flight responses in the face of environmental threats (Gianaros & Jennings, 2018; Ginty, Kraynak, Fisher, & Gianaros, 2017). For example, historically being able to efficiently enable the above changes might improve the chance of survival from an attack from a predator, however this analogy of survival is somewhat lost in the 21st century (Dhabhar, 2009). Rather, stress responses are engaged numerous of times during the day by situations that place demands on the body's resources and require a focussing of attention e.g., being criticised by your boss, or having your house broken into (Benschop & Schedlowski, 1999). Consequently individuals frequently experience stress responses which are not deemed necessary for the situation and this may have negative consequences for health.

There is considerable evidence supporting the relationship between the magnitude of an individual's acute stress response and adverse health outcomes such as cardiovascular disease (Chida & Steptoe, 2010; Kivimäki & Steptoe, 2018; Steptoe & Kivimaki, 2012), depression (Cohen, Janicki-Deverts, & Miller, 2007; O'Neill, Cohen, Tolpin, & Gunthert, 2004; Parrish, Cohen, & Laurenceau, 2011), and affective disorders. (Charles, Piazza, Mogle, Sliwinski, & Almeida, 2013). Chronic stress has also been related to adverse health outcomes including cardiovascular disease (Krantz & McCeney, 2002; Song et al., 2019), anxiety (Cohen, Edmondson, & Kronish, 2015; Heim & Nemeroff, 2001; Kahn & Khan, 2017) and depression (Hammen, 2005; Kahn & Khan, 2017; Paykel, 1976). In addition, literature implicates stress as a factor which can exacerbate symptoms in several diseases, including inflammatory bowel disease (IBD) (Camara, Ziegler, Begre, Schoepfer, & von Kanel, 2009),

rheumatoid arthritis (Yılmaz, Umay, Gündoğdu, Karaahmet, & Öztürk, 2017), cardiovascular disease (Rozanski, Blumenthal, & Kaplan, 1999) and cancer (Antoni et al., 2006). Taken together, in the 21st century stress has detrimental effects on the body, and consequently understanding factors which contribute to acute stress reactivity and whether techniques can be used to reduce the impact of stress is important for health and well-being.

This thesis aims to examine individual factors related to the physiological and psychological responses to acute psychological stress, and whether these stress responses can be altered through an acute intervention. Secondly, this thesis will explore whether stress is associated with disease symptoms in patients with ulcerative colitis (UC) a clinical population in which stress has been suggested to contribute to disease burden. From a health perspective, understanding determinants of individual differences in psychophysiological stress responses will aid in the ability to provide appropriate and targeted treatment to individuals susceptible to the negative outcomes resulting from stress.

## The Generation of Stress Responses

A fundamental assumption within the psychological stress literature is that when an individual is exposed to a stressful situation, the interaction between their personal evaluation of this event and the perception of coping resources is instrumental in the generation of a stress response (Lazarus, 1966; Lazarus & Folkman, 1984). An '*event*' is defined as either an external event (e.g., your work boss firing you), or an internal event (e.g., anticipation of an upcoming exam/job interview) which an individual appraises as personally demanding and relevant. A subsequent series of appraisals occurs, which can be categorised as primary and secondary appraisals. Primary appraisals are defined as the evaluation of the perceived demands of a situation, and secondary appraisals are defined as the evaluation of the

availability of perceived coping resources (Lazarus & Folkman, 1984). Central to models of psychological stress is that the psychological and physiological stress responses result from an interaction between the environment and an individual's appraisals (Gianaros & Jennings, 2018; Lazarus & Folkman, 1984; Mason, 1971). Consequently, there is a complex multi-factorial pathway beginning from an '*event',* which influences the appraisal processes and subsequent stressor-evoked responses. It is therefore not surprising that there is individual variability in these psychological and physiological stress responses.

**Cardiovascular Responses to Acute Psychological Stress**

Out of the physiological responses to stress, cardiovascular responses have been most extensively explored. It is well recognised that when exposed to stress, most people experience increases in heart rate (HR), cardiac output (CO), systolic blood pressure (SBP) and diastolic blood pressure (DBP) (AlAbsi et al., 1997; Beh, 1998; Brindle, Ginty, Phillips, & Carroll, 2014; Ginty et al., 2017; Kamarck & Lovallo, 2003; Turner, 1994), as a result of changes in autonomic function, specifically sympathetic and parasympathetic activity (Brindle et al., 2014; Brotman, Golden, & Wittstein, 2007; Cacioppo et al., 1994; Dampney, 2015; Flaa, Eide, Kjeldsen, & Rostrup, 2008; Porges, 1995; Salomon, Matthews, & Allen, 2000). As alluded to above, from an evolutionary perspective these responses may be adaptive in order to respond to stressful stimuli, however extensive literature supports an association between the magnitude of cardiovascular stress reactivity and negative health outcomes (Carroll, Ginty, Der, et al., 2012; Chida & Steptoe, 2010; Steptoe & Kivimaki, 2012).

This work has stemmed from seminal research from Paul Obrist, who formed the *cardiovascular reactivity hypothesis* which proposes that exaggerated cardiovascular

responses to acute psychological stress are predictive of negative health outcomes (Obrist, 1981). Substantial evidence supports the reactivity hypothesis, with work from cross-sectional as well as large-scale prospective studies demonstrating an association between stressor-evoked cardiovascular reactivity and future hypertension/blood pressure status (Carroll, Ginty, Der, et al., 2012; Carroll, Ring, Hunt, Ford, & MacIntyre, 2003; Carroll et al., 2001), markers of atherosclerosis (Barnett, Spence, Manuck, & Jennings, 1997; Everson et al., 1997; Kamarck et al., 1997), and cardiovascular mortality (Carroll, Ginty, Der, et al., 2012; Steptoe & Kivimaki, 2012). This work is further supported by meta-analyses showing a positive association between stressor-evoked cardiovascular reactivity and poorer future cardiovascular health status (Chida & Steptoe, 2010; Turner et al., 2019).

Interestingly, although stressor-evoked cardiovascular reactivity plays a contributory role towards negative health outcomes, exercise elicits a similar pattern of increases in HR, CO, SBP and DBP. However regularly undertaking exercise is commonly seen as beneficial for health, and can protect against physiological and psychological pathologies. The distinction between regular exercise and stress appears to be explained by the metabolically-exaggerated responses observed during stress compared to the tightly coordinated metabolically-appropriate response observed during exercise (Balanos et al., 2010; Carroll, Phillips, & Balanos, 2009). During exercise, there is a coordinated combination of top-down and bottom-up control over peripheral physiological responses (Mitchell, 1985; Williamson, Fadel, & Mitchell, 2006). That is, top-down influences over visceral control are met with feedback from working muscles, resulting in a metabolically appropriate physiological response (Kaufman, 2012; Williamson et al., 2006). In comparison, during stress, there is a metabolic uncoupling with perturbations in peripheral physiology observed without relative concurrent increases in metabolic expenditure (Balanos et al., 2010; Carroll et al., 2009). It is

this uncoupling and metabolically exaggerated stressor-evoked cardiovascular response, which is postulated to underlie the pathophysiological associations between stressor-evoked cardiovascular reactivity and health.

Although this metabolic uncoupling occurs, not all individuals experience large increases in cardiovascular parameters during acute psychological stress, and some even experience very little or no change (Phillips, Ginty, & Hughes, 2013). Evidence has grown to support what is commonly referred to as "blunted reactivity" in which comparatively lower than usual cardiovascular reactivity is observed (de Rooij, 2013; Ginty, 2013; Ginty, Gianaros, Derbyshire, Phillips, & Carroll, 2013; Phillips et al., 2013). Research examining blunted reactivity suggests these low cardiovascular reactions to acute psychological stress are associated with adverse health outcomes such as depression (Carroll, Phillips, Hunt, & Der, 2007; Phillips, Hunt, Der, & Carroll, 2011) and poor self-reported health (Phillips, Der, & Carroll, 2009). As such, understanding factors that determine an individual's cardiovascular response is of interest. One particularly interesting hypothesis to explore is whether dispositional factors influence cardiovascular reactivity. Specifically, identifying biological markers that may predispose individuals to experience either heightened or blunted stress reactivity could result in early identification and prevention of future health problems.

**The Role of the Brain in Acute Psychological Stress**

As outlined, there is large individual variability in the magnitude and pattern of stressor-evoked cardiovascular responses. Understanding reasons for these individual differences has been a central question driving the cardiovascular stress literature for decades. Given the fundamental role of the brain in the interpretation of external and internal events, as well as organising descending projections to control peripheral physiology (Gianaros, Sheu, et

al., 2017; Wager, van Ast, et al., 2009; Wager, Waugh, et al., 2009), brain circuits implicated in both processes could play an integral role underlying individual differences in stress reactivity (Lovallo, 2005; Lovallo & Gerin, 2003). With advancements in neuroimaging techniques, it is possible to explore how the brain is related to stress reactivity. In particular, functional magnetic resonance imaging (fMRI) assesses changes in blood oxygenation on a second by second basis, and provides detailed measurements of brain activation (Glover, 2011). This allows an understanding of how activity in brain structures (e.g., the amygdala) relates to cognitive and behavioural functions such as the generation of an appraisal or an increase in heart rate. In addition, structural neuroimaging provides a detailed assessment of the morphology (i.e., grey matter volume and shape) of the brain. This is particularly interesting from a clinical health perspective, as the morphology of brain structures may play an important role in the regulation of behavioural functions.

Several studies have begun to shed light on the complex interaction between functional activity of brain structures and how these regions stimulate visceral activity during stress. Indeed, literature has demonstrated that corticolimbic brain circuits appear to be closely involved in both the processing and appraisal of stressful situations and in the generation and control of stressor-evoked cardiovascular reactivity (Gianaros & Jennings, 2018; Ginty et al., 2017; Lovallo & Gerin, 2003; Ulrich-Lai & Herman, 2009; Wager, van Ast, et al., 2009; Wager, Waugh, et al., 2009). Specifically, functional activity of the amygdala and hippocampus have consistently been shown to be associated with cardiovascular activity during stress (Beissner, Meissner, Bar, & Napadow, 2013; Critchley, 2005, 2009; Myers, 2017). One particular study investigating the role of the amygdala and hippocampus structures in stress reactivity was Gianaros et al. (2008). It was found that during acute psychological stress, individuals who expressed greater mean arterial blood

pressure (MAP) reactivity, displayed greater amygdala and anterior cingulate cortex (ACC) functional activity (Gianaros et al., 2008). In addition, connectivity analyses revealed the MAP reactivity varied with greater amygdala-pons and amygdala-pACC functional connectivity suggesting that the amygdala might act as an important regulator of cardiovascular reactivity, signalling via relay pathways to autonomic control regions (Gianaros & Sheu, 2009; Gianaros et al., 2008; Ginty et al., 2017). As such, functional activity in these important limbic brain structures appears to relate to individual differences in cardiovascular reactivity, however the role and importance of the morphology (i.e., the grey matter volume) of these structures in acute psychological stress has received significantly less attention.

Several lines of work from anxiety, post-traumatic stress disorder (PTSD), and chronic stress literature have found associations between chronic stress and amygdala morphology (Aghajani et al., 2016; Lakshminarasimhan & Chattarji, 2012; Pezawas et al., 2005; Pietrzak et al., 2015; Yang et al., 2008) as well as hippocampus morphology (Butterworth, Cherbuin, Sachdev, & Anstey, 2012; Gerritsen et al., 2015; Gilbertson et al., 2002; Herman, McKlveen, Solomon, Carvalho-Netto, & Myers, 2012; Kremen, Koenen, Afari, & Lyons, 2012; Lakshminarasimhan & Chattarji, 2012; Lindgren, Bergdahl, & Nyberg, 2016; Lyons, Yang, Sawyer-Glover, Moseley, & Schatzberg, 2001). These studies highlight that amygdala and hippocampal morphology and the expression of stress-related disorders are closely related, and that these brain structures could be an important biological factor which determines an individual's experience of stress. This is further supported by literature demonstrating ablation of the amygdala in rats reduces the cardiovascular response to stress, suggesting the amygdala plays a direct mediating role between stress and cardiovascular reactivity (Sanders, Wirtz-Nole, DeFord, & Erling, 1994).

Only one study has explored whether cardiovascular reactivity to acute psychological stress is associated with brain morphology with only the amygdala being investigated (Gianaros et al., 2008). In this study, MAP reactivity was found to negatively associate with amygdala grey matter volume. That is, greater MAP reactivity during stress was associated with lower amygdala grey matter volume (Gianaros et al., 2008). Interestingly, negative correlations were observed between amygdala volume and amygdala activity during stress. When controlling for amygdala grey matter volume, the correlation between MAP reactivity and amygdala activity was no longer significant. This suggests that amygdala morphology may be important in the regulation of stressor-evoked cardiovascular activity (Gianaros et al., 2008). This poses interesting questions regarding the importance of brain morphology in influencing cardiovascular reactivity during acute psychological stress. As such, while evidence implicates corticolimbic brain structures being related to chronic measures of stress, there is a lack of evidence examining whether these brain structures, essential in stress appraisal, regulation of autonomic control, and behavioural responding, are associated with stressor-evoked cardiovascular reactivity. Identifying whether brain morphology plays an integral role in cardiovascular stress reactivity will allow an understanding of biological markers which could underlie individual differences in stressor-evoked cardiovascular reactivity.

**Psychological Responses to Acute Psychological Stress**

Whilst the cardiovascular responses to acute stress have received substantial attention, the psychological responses have received somewhat less emphasis, yet may confer equal importance to health. Given the high prevalence of mental health issues in society, and the associations between chronic stress and psychiatric disorders (Cohen, Edmondson, &

Kronish, 2015; Kahn & Khan, 2017), it is surprising that the role of individual differences in psychological responses to acute stress has received little attention. Indeed, it is well known that during acute psychological stress, individuals experience alterations in the subjective experience of stress, typically demonstrated as increases in anxiety (AlAbsi et al., 1997; Bosch et al., 2009; Grossman, Wilhelm, Kawachi, & Sparrow, 2001), negative affect (Feldman, Cohen, Hamrick, & Lepore, 2004; Feldman et al., 1999; Hilmert & Kvasnicka, 2010) and the reporting of perceived stress (Schlotz, Hammerfald, Ehlert, & Gaabb, 2011; Schlotz et al., 2008). However, less research has focussed specifically on individual factors determining the psychological responses during stress. This is important, as the experience of stress-induced psychological changes may lead to the development of adverse psychological outcomes (Charles et al., 2013).

Indeed, studies using daily diaries have reported associations between negative affect experienced during daily stressors and greater depression symptoms 2-months later (Parrish et al., 2011). Similarly, longitudinal analyses from the Midlife Development in the United States (MIDUS) study reported associations between negative affect during daily stressors over an eight day period and general negative distress as well as self-reported affective disorder 10-years later (Charles et al., 2013). Increased negative affect to minor life stressors has also been proposed to relate to mental disorders, where greater emotional reactivity to daily stress is suggested to relate to an increased vulnerability of developing psychosis (Myin-Germeys & van Os, 2007). Further, Gomes, Faria, and Lopes (2016) in an observational study of 2000 nurses explored how work-related stress associated with psychological health. It was found that stress appraisals mediated the relationship between daily occupational stress and psychological health. Specifically, stress and perceptions of threat were positively related to mental health problems, whereas appraisals including perceived coping ability and perceived

control were negatively related to mental health problems (Gomes et al., 2016). Taken together, negative psychological states experienced during stress may be of clinical relevance, leading to negative psychological health outcomes. Examining factors that contribute to these changes in psychological state during periods of acute stress are therefore important to help understand why some individuals experience greater emotional stress and may be at increased risk of future negative psychological outcomes.

One of the most frequently reported psychological responses examined during acute psychological stress is anxiety (AlAbsi et al., 1997; Anderson & Hope, 2009; Bosch et al., 2009; Paine, Watkins, Blumenthal, Kuhn, & Sherwood, 2015). Anxiety is a multidimensional construct comprised of distinct cognitive and somatic components (Degood & Tait, 1987; Martens, Burton, Vealey, Bump, & Smith, 1990; Morris, Davis, & Hutchings, 1981). Cognitive anxiety refers to the mental component and includes symptoms such as negative expectations, worries, and concerns, whereas somatic anxiety refers to the physiological-affective anxiety symptoms including changes in autonomic arousal (Mellalieu, Hanton, & Fletcher, 2006; Morris et al., 1981).

Anxiety has historically been considered a negative emotion and thus researchers have typically considered its presence to have a detrimental effect on coping and performance. However, over the last 20 years, numerous studies demonstrate that when anxiety is present during competitive situations, it is not always considered negative towards coping or performance (Jones, 1995; Jones, Hanton, & Swain, 1994; Jones, Swain, & Hardy, 1993; Jones & Swain, 1992; Mellalieu et al., 2006). Indeed, these studies showed that greater levels of anxiety can actually be perceived as beneficial. Consequently, anxiety can vary both in terms of the magnitude of symptoms (i.e., its intensity), as well as whether or not these symptoms are perceived as facilitative or debilitative (i.e., it's perceived interpretation).

Importantly, the perceived interpretation of anxiety symptoms can also be a stronger predictor of performance than the intensity of the anxiety (Chamberlain & Hale, 2007; Swain & Jones, 1996). It is therefore essential for researchers to assess both the intensity and perceived interpretation of cognitive and somatic anxiety, as two individuals with a similar 'overall' anxiety intensity score may differ dramatically in the separate levels of cognitive and somatic anxiety intensity and their perceived interpretation of these symptoms which could influence their coping ability and performance during stress.

Yet despite evidence in the literature highlighting the importance or assessing the different anxiety components, studies investigating the psychological responses to acute psychological stress typically use general mood questionnaires such as the Profile of Mood States which computes a general overall anxiety score (McNair, 1984; McNair, Lorr, & Droppleman, 1971). Consequently a detailed understanding of factors that contribute to the expression of cognitive and somatic anxiety intensity and perceived interpretation during acute psychological stress is relatively sparse. This is particularly interesting, given that during acute psychological stress, both cognitive-based responses (i.e., appraisals, negative mood) and somatic-based responses (i.e., heart rate, blood pressure) occur simultaneously. Thus, further investigation of personal factors that contribute towards the experience of cognitive and somatic anxiety is of interest. Specifically, a greater understanding of whether specific psychological and/or physiological stress responses relate differentially with the multidimensional constructs of anxiety is needed in order to better understand factors increasing the risk of developing anxiety disorders.

**Physiological and Psychological Response Coherence During Acute Psychological Stress**

During acute psychological stress individuals experience concurrent psychological and physiological perturbations and it is appealing to suggest that these two systems are tightly coupled. It has long been thought that there is a close relationship between the psychological and physiological stress axis, even in the 19th century Charles Darwin suggested that negative emotions directly influence the cardiovascular system (Darwin, Cummings, Duchenne, & John, 1872). However, there is mixed evidence supporting the concept of response coherence during acute psychological stress. Two meta-analyses explored the relationship between physiological and psychological responses to stress (Campbell & Ehlert, 2012; Feldman et al., 1999). First, Feldman et al. (1999) reported weak associations between cardiovascular reactivity and overall emotional experience (negative emotion, anger and anxiety), with emotion accounting for between 2% and 12% of the variance in cardiovascular responses. Second, Campbell and Ehlert (2012) reported that only 25% of studies demonstrated significant associations between measures of HR reactivity and psychological responses (primarily general anxiety measures). However, both the Feldman et al. (1999) and Campbell and Ehlert (2012) meta-analyses included studies that used unidimensional measures of anxiety from questionnaires including the POMS (McNair, 1984), Stress/Arousal Adjective Checklist (King, Burrows, & Stanley, 1983), State and Trait Anxiety Inventory (Spielberger, Gorsuch, & Lushene, 1970), and single item Likert scales generated specifically for the studies. Studies may have demonstrated limited associations between physiological states and negative emotional responses to stress due to not implementing multidimensional psychological assessments i.e., both cognitive and somatic anxiety intensity as well as perceived interpretation. Research is therefore needed to understand the variability in the

psychological responses to stress, and examine factors contributing towards individuals experiencing greater levels of psychological stress reactivity.

**Exercise and Stress Reactivity**

As articulated above, a range of physiological and psychological stress responses are induced during acute psychological stress, and there are individual differences in these responses. In addition to individual factors, situational factors can alter the magnitude of stress response. For example, in a meta-analysis of cardiovascular reactivity, the magnitude of cardiovascular and autonomic activity varied across speech, mental arithmetic and reaction time tasks (Brindle et al., 2014). Studies have also demonstrated the length of task (Ring, Burns, & Carroll, 2002) and level of social evaluation can influence cardiovascular reactivity (Bosch et al., 2009). These findings demonstrate that reactivity to stress is not a fixed general response, but rather a complex interaction between the environmental situation and individual factors. As such, whether an individual's stress response can be altered through changing environmental stimuli is of great interest. One particular modifiable behaviour that could have benefits for stress reactivity is exercise. Exercise is a physical stressor, inducing cardiovascular responses in order to meet the required metabolic demands (McArdle, Katch, & Katch, 2001) and substantial empirical research implicates regular exercise as having beneficial effects on health (Penedo & Dahn, 2005; White et al., 2017).

Engaging in acute exercise has also been shown to be beneficial for mental health by inducing positive mood states and reducing general stress levels (Maroulakis & Zervas, 1993; Reed & Ones, 2006; Sibold & Berg, 2010). Sibold and Berg (2010) demonstrated that 20-mins of aerobic cycling at 60% $VO_{2max}$ was effective at improving mood (reduced total mood disturbance) immediately following the exercise, and up to 12-hours post-exercise. Beneficial

effects of exercise have also been observed in patients with psychological disorders. For example in patients with depression, 30-mins of cycling at 60-70% of maximal or 30-mins of quiet rest were both effective at reducing mood, assessed with the POMS. However in the exercise group only, increases in positive well-being as assessed with the subjective exercise experiences scale were evident (Bartholomew, Morrison, & Ciccolo, 2005). As such, using an acute bout of exercise is potentially an effective methodology to improve mood and reduce perceptions of stress (Basso & Suzuki, 2017). However, it is unclear whether an acute bout of exercise prior to psychological stress has beneficial effects on the typically negative psychological responses to stress. If individuals who are aware of an upcoming psychological stressor could use an acute bout of exercise to attenuate the negative responses of stress, this could be a valuable technique to alleviate the negative impact of stress.

Several lines of research have explored whether acute exercise can alter the cardiovascular stress response to acute psychological stress. It has consistently been shown that following an acute bout of exercise, blood pressure responses to acute psychological stress are reduced in healthy participants (Alderman, Arent, Landers, & Rogers, 2007; Brownley et al., 2003; Hamer, Taylor, & Steptoe, 2006; Rejeski, Thompson, Brubaker, & Miller, 1992; Roy & Steptoe, 1991) as well as patients with hypertension (Gauche et al., 2017). These findings were summarised in a meta-analysis which found in 10/15 studies following exercise, there was a mean reduction in SBP of 3.7mmHg and a reduction in DBP of 3.0mmHg during stress, independent of physical fitness (Hamer et al., 2006). Importantly, moderate to high intensity exercise is thought to have a larger effect on blood pressure attenuation, with an inverse relationship between exercise intensity before stress and subsequent cardiovascular reactivity (Alderman, Arent, Landers, & Rogers, 2007).

Consequently, an acute bout of exercise (i.e., 10-20mins) prior to a stressful event appears to have beneficial effects on the cardiovascular reactivity experienced during stress.

Significantly less research has investigated how an acute bout of exercise impacts the psychological response to acute psychological stress. As stressful experiences result from the interaction between an individual's perception of an event and their coping resources, if an individual experienced reduced physiological stress reactivity, it is likely that less debilitating psychological responses would concurrently be experienced. Although, the coherence evidence provided above demonstrates weak associations between physiological and psychological responses and as such, it is not clear whether similar beneficial effects will be observed for psychological outcomes. If psychological responses are not improved or even worsened, the validity of using high intensity exercise as a stress reduction technique must be examined. Health must be considered as both physical and mental, and although blood pressure reactivity may be attenuated, if negative psychological states during stress are exaggerated, this has implications for mental health.

The few studies that have investigated the role of acute exercise on the psychological responses have only included psychological responses as secondary outcomes, providing mixed results (Duda, Sedlock, Melby, & Thaman, 1988; McGowan, Robertson, & Epstein, 1985; Roy & Steptoe, 1991; Steptoe, Kearsley, & Walters, 1993). For example, no effects on psychological responses (Duda et al., 1988; McGowan et al., 1985; Roy & Steptoe, 1991), decreases in anxiety (Roth, 1989), as well as increases in anxiety and depression (Steptoe et al., 1993) have been reported following an acute bout of exercise. Furthermore, psychological measures in the above studies only assessed mood states with the POMS and a complete understanding of the effects of exercise on psychological states is not evident. Given the multi-bodily systems involved in exercise and stress, a detailed assessment based on the

transactional model of stress and coping (Lazarus & Folkman, 1984) including psychological appraisals and the multidimensional constructs of anxiety would be an insightful investigation to whether exercise alters the psychological response to stress.

**The Role of Stress in Inflammatory Bowel Disease**

The current thesis has outlined that psychological stress is related to the development of a range of adverse health outcomes including debilitating disease states. Psychological stress as also been implicated in the exacerbation of symptoms in numerous diseases. One such pathology where individual differences in stress reactivity are thought to play a central role in worsening active symptoms is inflammatory bowel disease (IBD). Inflammatory bowel disease is a gastrointestinal disorder including ulcerative colitis (UC) and Crohn's disease (CD) with prevalence in European countries reported as 505 and 322 per 100,000, respectively (Ng et al., 2017). Inflammatory bowel disease is most commonly diagnosed in 20 – 30 year olds but can affect individuals at any age and is characterised by clinical symptoms including diarrhoea, mucus and blood in stools, abdominal pain, nausea, weight loss and fatigue (Ko & Auyeung, 2014). The aetiology of IBD is not clear, but both environmental and genetic factors are proposed to influence the development of the disease. With no known cure, the medical goal is to reduce symptom burden by reducing inflammation with medical therapies including corticosteroids, aminosalicylates, immuno-modulators, antibiotics, anti-tumor necrosis factor-α (TNF-α) and probiotics among other therapeutic options (Kozuch & Hanauer, 2008). The disease course fluctuates unpredictably between relapsing and remitting disease states which has a significant impact on patient health and quality of life. The notion that stress alters gastrointestinal symptoms is not new, however only recently has this topic received increased clinical attention (Bernstein, Walker, & Graff, 2006; Brzozowski et al.,

2016; Maunder & Levenstein, 2008; Sajadinejad, Asgari, Molavi, Kalantari, & Adibi, 2012; Triantafillidis, Merikas, & Gikas, 2013). While a growing number of studies have investigated the associations between perceived life stress and IBD symptoms, demonstrating a bi-directional association (Sexton et al., 2017; Targownik et al., 2015), the exact mechanisms through which stress contributes to the exacerbation of symptoms is not clear.

One mechanism which is appealing to explain the connection between stress and symptom exacerbation and has received significant clinical interest is the pathway called the 'brain-gut axis'. The brain-gut axis involves complex bidirectional interactions between central neural networks, the autonomic nervous system, and enteric nervous system (Bonaz & Bernstein, 2013; Brzozowski et al., 2016; Hollander, 2003). Indeed, several pathways have been suggested to play an integral role in the relationship between stress and IBD activity. For example, when exposed to stress there is activation of the sympathetic nervous system which promotes pro-inflammatory signalling pathways and mast cell activation (Brzozowski et al., 2016). In contrast, stress inhibits the vagus nerve activity, which has anti-inflammatory effects (Bonaz, Bazin, & Pellissier, 2018; Pavlov, Wang, Czura, Friedman, & Tracey, 2003). There is convincing evidence in healthy populations that stress stimulates inflammatory pathways, primarily indicated with increases in cytokines in the circulation (Marsland, Walsh, Lockwood, & John-Henderson, 2017; Rohleder, 2019; Steptoe, Hamer, & Chida, 2007). Specifically, Steptoe et al. (2007) highlighted that psychosocial stress promotes a significant increase in IL-6 and IL-1β, which was recently supported by Marsland et al. (2017), replicating large increases in IL-6, and IL-1β, but also IL-10 and TNF-α. As such, patients with IBD may experience an exacerbation of symptoms during periods of heightened stress due to greater inflammation, yet the associations between stress and IBD symptomology is relatively understudied.

Early work by Duffy et al. (1991) demonstrated an association between stressful life events and IBD disease activity in a mixed sample of CD and UC patients. Similar associations between perceived stress and self-report disease activity have been reported in UC patients (Bernstein et al., 2006; Bitton, Sewitch, Peppercorn, Edwardes, & Shah, 2003; Levenstein, 2003) and CD patients (Bernstein et al., 2006; Bitton et al., 2008). Indeed, in a review of 20 studies, 13 studies reported associations between stress and adverse IBD-related outcomes. However, the above studies measure disease activity with self-report measures and understanding whether stress directly influences inflammation which is of central importance to classification of the disease, is important for understanding how to clinically manage disease symptoms.

More recently, large-scale ($n$ = 478) cross-sectional (Targownik et al., 2015) as well as prospective (Sexton et al., 2017) studies have shown that perceived stress significantly associates with greater reported disease symptoms (Targownik et al., 2015). Participants also provided a stool sample to assess faecal calprotectin (FCAL), a bowel specific marker of inflammation. Interestingly, perceived stress was unrelated to intestinal inflammation in both CD and UC patients in both studies (Sexton et al., 2017; Targownik et al., 2015). Further, bowel inflammation was not associated with symptom activity in CD, and was only weakly associated in UC patients. This suggests that stress and self-report symptoms appear to be closely related, yet the relationship may occur independently of intestinal inflammation. Examining how patients with IBD respond to acute stress, and whether they demonstrate an altered physiological or psychological response, could help explain the relationship between stress and disease symptoms.

The use of acute experimental laboratory-based stress provides an innovative paradigm in IBD to explore this research question. To our knowledge, only two studies have

explored the response to acute psychological stress in patients with IBD (Kuroki et al., 2011; Mawdsley, Macey, Feakins, Langmead, & Rampton, 2006). First, in patients with UC and healthy controls, no group differences were observed at baseline or for stressor-evoked changes in pulse rate, systolic and diastolic blood pressure, serum interleukin-6 (IL-6) or IL-13 (Mawdsley et al., 2006). However, it must be noted the stress task used was a 50-min dichotomous listening IQ test and no stress-induced changes in serological inflammatory markers were observed, raising concerns over the validity and effectiveness of the stress task. Second, in a mixed UC and CD sample and healthy control sample, no group differences were observed in physiological responses to stress, however IL-6 was greater at baseline and stress-induced alterations in IL-6 were observed in the IBD group only (Kuroki et al., 2011).

Taken together, it is currently not known if patients with IBD respond to stress with an altered stress response and whether stress influences self-reported disease activity as well as objective disease activity, i.e., inflammation. Consequently there is a need to take a multi-disciplinary approach to examine the complex interactions between stress and disease activity in patients with IBD.

**The Present Thesis**

Acute psychological stress elicits both physiological and psychological responses to stress, which have been implicated in the development of negative health outcomes. There is a need for understanding factors that contribute to the individual differences in these responses, which will ultimately inform the development of interventions, reducing the maladaptive effects of stress on the mind and body. Accordingly, the following four empirical studies explored factors which are associated with both the physiological and psychological responses to acute psychological stress in healthy and patient populations.

First, given the central role of the brain in mediating stressor-evoked cardiovascular reactivity, the aim of Chapter 2 was to explore whether dispositional individual differences in brain morphology were associated with detailed measures of stressor-evoked cardiovascular reactivity. To examine this research question, healthy participants completed an acute psychological stress task whilst concurrent cardiovascular activity was measured with electrocardiography, Doppler echocardiography and a blood pressure monitor to assess HR, SBP, DBP, MAP, stroke volume (SV), CO and total peripheral resistance (TPR). A subsequent MRI scan was completed to assess brain morphology. It was hypothesised that greater levels of stressor-evoked cardiovascular reactivity would associate with lower amygdala and hippocampus grey matter volume.

Evidence from Chapter 2 demonstrated individual differences in cardiovascular reactivity are associated with brain morphology which may predispose some individuals to a greater susceptibility of experiencing stress reactivity. Chapter 3 explored whether engaging in an acute bout of exercise could alter stressor-evoked responses. Extending from previous research (Hamer et al., 2006), an acute bout of exercise was used to attenuate the cardiovascular responses to stress. Exercise is also proposed to have beneficial psychological responses yet few studies have investigated the effects of an acute bout of exercise on psychological responses to stress. As such, Chapter 3 used a repeated measures experimental design to investigate the impact of engaging in an acute bout of exercise (10-mins) prior to psychological stress on cardiovascular responses (HR, SBP, DBP) as well as detailed psychological measures including appraisals, anxiety and mood responses to stress in healthy participants. It was hypothesised that a short bout of high intensity exercise would attenuate cardiovascular responses, but have a negative impact on psychological states during stress.

There is a deficiency of studies examining what factors contribute towards negative outcomes such as anxiety experienced during acute psychological stress. As such, Chapter 4 explored whether HR changes or perceptions of HR changes during stress were more strongly associated with cognitive and somatic anxiety intensity and perceived interpretation of these symptoms. Using a two-study approach, males (study 1) and females (study 2) completed three acute psychological stress tasks while HR was measured with electrocardiography, and self-reported perceived HR change and anxiety were assessed. It was hypothesised that there would be dissociation between actual HR and anxiety measures, yet perceived change in HR would be associated with anxiety intensity during stress.

The first three studies explored factors associated with stress reactivity in healthy individuals highlighting novel factors influencing cardiovascular and psychological responses to acute psychological stress. The final empirical study of the thesis (Chapter 5) explored the role of stress reactivity in patients with UC. Ulcerative colitis is a disease characterised by fluctuating disease symptoms thought to be associated with perceived stress. However, the role of acute stress in contributing to the reporting of symptom burden has not been explored in detail. Using a cross-sectional study design, patients with UC and healthy controls completed an acute psychological stress task whilst having physiological and psychological responses assessed. Measures of life stress and disease burden in UC patients were also reported. It was hypothesised that compared to healthy controls, there would be no differences in stress reactivity in UC patients, but stress responses in the laboratory would be associated with UC symptom burden.

# CHAPTER 2

## INCREASED STRESSOR-EVOKED CARDIOVASCULAR REACTIVITY IS ASSOCIATED WITH REDUCED AMYGDALA AND HIPPOCAMPUS VOLUME

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## Abstract

Exaggerated cardiovascular reactivity to acute psychological stress is associated with an increased risk of developing cardiovascular disease. The amygdala and hippocampus have been implicated in centrally mediating stressor-evoked cardiovascular reactivity. However, little is known about the associations of amygdala and hippocampus morphology with stressor-evoked cardiovascular reactivity. Forty (*M* [*SD*] age = 19.05 [0.22] years) healthy young women completed two separate testing sessions. Session one assessed multiple parameters of cardiovascular physiology at rest and during a validated psychological stress task (Paced Auditory Serial Addition task), using electrocardiography, Doppler echocardiography and blood pressure monitoring. In session two, one year later, structural MRI was conducted. Brain structural volumes were computed using automated segmentation methods. Regression analyses, following Benjamini-Hochberg correction, showed that greater heart rate and cardiac output reactivity were associated with reduced amygdala and hippocampus grey matter volume. Systolic blood pressure reactivity was associated with reduced hippocampus volume. In contrast, no associations between diastolic blood pressure, mean arterial blood pressure, stroke volume, or total peripheral resistance reactivity with amygdala or hippocampus volumes were apparent. Comparison analyses examining insula volume found no significant associations. Some indicators of greater stressor-evoked cardiovascular reactivity associate with reduced amygdala and hippocampus grey matter volume, but the mechanisms of this association warrant further study.



Key words: Amygdala; Hippocampus; Psychological stress; Stressor-evoked cardiovascular reactivity; Brain morphology; Individual differences; Magnetic resonance imaging

## Introduction

The reactivity hypothesis postulates that exaggerated cardiovascular reactivity to acute psychological stress predisposes individuals to risk for cardiovascular disease (Manuck, Kasprowicz, & Muldoon, 1990; Obrist, 1981). Evidence consistent with this hypothesis has been derived from cross-sectional and prospective studies showing that higher levels of stressor-evoked cardiovascular reactivity are associated with future blood pressure status and hypertension (Carroll, Ginty, Painter, et al., 2012), atherosclerosis (Barnett et al., 1997), and cardiovascular disease mortality (Carroll, Ginty, Der, et al., 2012). Indeed, a meta-analysis of 36 studies showed greater stressor-evoked cardiovascular reactivity to associate with adverse future cardiovascular health status (Chida & Steptoe, 2010).

Considerable effort has been put into examining the bases of stable individual differences in stressor-evoked cardiovascular reactivity. Evidence from neuroimaging studies has extended prior animal evidence to implicate neural systems as partial mediators of individual differences in stressor-evoked cardiovascular reactivity (Gianaros & Sheu, 2009; Gianaros, Sheu, et al., 2017; Gianaros & Wager, 2015; Ginty et al., 2017; Wager, van Ast, et al., 2009). For example, human neuroimaging studies demonstrate that the hippocampus and amygdala are limbic structures related to autonomic cardiovascular control during stress (e.g., Gianaros & Wager, 2015; McEwen & Gianaros, 2011) and a recent review has suggested that these brain structures may influence stressor-evoked cardiovascular reactivity via predictive neural processes that calibrate physiology with anticipated behavioural demands (Gianaros & Jennings, 2018). Although it is known that stressor-evoked cardiovascular reactivity is associated with concurrent activation in these corticolimbic systems (Gianaros & Sheu, 2009; McEwen & Gianaros, 2011; Myers, 2017; Wager, Waugh, et al., 2009), there is substantially less research examining if underlying morphological differences of the hippocampus and amygdala are associated with individual differences in cardiovascular stress reactivity. Recent

work supports the importance of examining the relationship between brain morphology and cardiovascular autonomic function, where results have found high frequency heart rate variability (indicative of cardiac vagal function) to be negatively associated with grey matter volumes of striatal and limbic structures (e.g., amygdala) of the central autonomic network (Wei, Hong, & Wu, 2018).

To our knowledge, only one study has directly examined the associations between stressor-evoked cardiovascular reactivity and brain morphology in humans (Gianaros et al., 2008). Thirty-two healthy participants underwent a structural magnetic resonance imaging (MRI) scan. They then completed a standard stress task while functional activation of the brain and mean arterial pressure were measured simultaneously. Greater stressor-evoked mean arterial pressure reactivity was associated with lower amygdala grey matter volume as well as greater amygdala activity. The relationship between amygdala activation and stressor-evoked mean arterial pressure reactivity statistically depended on grey matter volume of the amygdala. Secondary analyses found greater stressor-evoked mean arterial pressure reactivity also associated with areas extending into the hippocampus (Gianaros et al., 2008). Thus, the amygdala and hippocampus volume appear to be involved in blood pressure reactivity to psychological stress. However, it is unknown if these associations are specific to blood pressure reactivity, or whether the hippocampus and amygdala are associated with other cardiovascular parameters.

The aim of the present study was to extend the previous study demonstrating greater stressor-evoked pressure reactivity being associated with reduced amygdala and hippocampus grey matter volume (Gianaros et al., 2008) by including more comprehensive measures of cardiovascular activity (i.e., heart rate, stroke volume, cardiac output, total peripheral resistance, systolic and diastolic blood pressure, and mean arterial pressure). Additional

analyses using a comparison region (the insula) were conducted to determine whether any significant associations were relatively specific to the amygdala and hippocampus. The insula was selected as the comparison region because it is functionally implicated in visceral control, specifically in the regulation of cardiovascular and autonomic function (Beissner et al., 2013; Oppenheimer & Cechetto, 2016). Prior human imaging work, however, has not identified an association between insular morphology and stressor-evoked cardiovascular reactivity. Therefore, it would not be expected to correlate with stressor-evoked cardiovascular reactivity. We hypothesised that higher levels of stressor-evoked cardiovascular reactivity would be associated with reduced volumes of the amygdala and hippocampus, but that there would no association between cardiovascular reactivity and insula volume.

## Method

### Participants

Participants were 40 healthy women (Mean age = 19.05, *SD* = 0.22 years; see Table 2.1 for demographics), participating in the Stress and Transitions to University (STUN) study (STUN study; Ginty, Brindle, & Carroll, 2015). The original STUN study included 81% women. The main aim of the STUN study was to examine how physiological responses to acute psychological stress predicted adaptation to college. A subsample of participants was recruited for this neuroimaging protocol. Only women were recruited for this small study using a subsample of the original sample due to the skew in gender in the original study. None of the participants had a history of cardiovascular disease or were smokers. All were asked to abstain from alcohol and vigorous exercise for 12h, caffeine for 2hr, and food and drinks other than water for 1hr before testing. All participants provided written consent prior to testing and received £10 for study participation. The study was approved by the University Ethics Committee.

**Table 2.1.** Mean (*SD*) participant demographics

| | Mean (*SD*) |
|---|---|
| Age | 19.05 (0.22) |
| BMI | 23.13 (4.73) |
| | No. (%) |
| Ethnicity | |
| White | 24 (60) |
| Black | 4 (10) |
| Other | 12 (30) |
| Socio-economic Status | |
| Professional | 16 (40) |
| Managerial | 8 (20) |
| Skilled Non-Manual | 7 (17.5) |
| Skilled Manual | 1 (2.5) |
| Partly Skilled | 3 (7.5) |
| Unskilled | 5 (12.5) |

**Cardiovascular Measurements**

Heart rate (HR) was measured continuously by electrocardiography (ECG) with electrodes (CONMED Corp, NY, USA) placed in a three lead configuration. Raw ECG data were collected using a Grass P511 amplifier (Grass Instruments, USA), CED Power1401 digital to analog converter, and Spike 2 software at a sampling frequency of 1000Hz. Using Kubios HRV, individual traces were visually inspected and any artefacts were removed. Average HR was calculated for each period (baseline, stress) using the full 10-min of data from each phase. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were measured using a semi-automatic oscillatory blood pressure monitor (Dinamap Pro 100; Critikon Inc., Tampa, FL) at minutes 2, 4, 6, and 8 of baseline and task periods. Stroke Volume (SV, ml), was assessed using a Phillips Sonos 7500 ultrasound machine with an S3 two-dimensional transducer (1-2 MHz). Flow through the aortic valve during systole was identified with an apical five-chamber view of the heart using

Doppler. The velocity profile of the aortic flow was obtained using pulsed-wave spectral mode at a screen sweep speed of 100mms−1. Doppler sampling of the flow was taken immediately below the orifice of the aortic valve. The flow was quantified automatically using the velocity time integral, which is the mean distance through which blood travels in the outflow tract during ventricular contraction. Each measurement of velocity time integral was made from at least three velocity profiles. The diameter of the aortic valve was measured from a parasternal long axis view and the aortic valve area was calculated. Cardiac output was calculated as HR x SV and is reported in litres/minute. Total peripheral resistance (TPR; dyne-s/$cm^{-5}$) was calculated using the formula, TPR = (MAP / CO) x 80.

**Acute Psychological Stress Task**

Participants completed a 10-min version of the Paced Auditory Serial Addition task (PASAT; Gronwall, 1977) whereby a series of single-digit numbers were presented through audio speakers. Participants were asked to add consecutive numbers together and verbalise their answers, while remembering the most recent number in order to add it to the next presented number. The PASAT involves social evaluation; participants were videotaped and they were informed that 'body language experts' would assess their anxiety levels. In reality, no such body language analysis took place. Additionally, a mirror was placed directly in front of the participant and they were instructed to watch themselves for the duration of the test. Finally, participants were informed they would hear a loud buzzer if they hesitated or answered incorrectly. Together, these elements of social evaluation, increased time pressure and punishment have been shown to increase self-reports of stress (Veldhuijzen Van Zanten et al., 2004). The PASAT reliably evokes cardiovascular reactivity (Ring et al., 2002; Veldhuijzen Van Zanten et al., 2004) and yields acceptable test-retest reliability in multiple parameters of physiology (Ginty et al., 2013).

## Procedures

One hundred and eighty five participants were briefed on the general protocol and provided informed consent after any questions had been answered. Firstly, a brief questionnaire pack was completed to collect demographic and other psychosocial information. Participants were asked to provide information about the occupational status of the parent who was the primary household provider, with options ranging from professional (e.g., doctor/lawyer) to unskilled worker (e.g., day labourer). Answers were then weighted from lowest to highest on a scale of 1 = unskilled to 6 = professional. This variable was taken to reflect parental socioeconomic status (SES). Height and weight were assessed, and BMI was computed as weight (kg) / height $(m)^2$. As part of the psychophysiology protocol, participants were asked to lie in a semi lateral decubitus position on a hospital bed, with supportive pillows for comfort. ECG spot electrodes were attached, as well as a blood pressure cuff over the brachial artery. The Doppler echocardiography probe was positioned to check image quality. Following a 10-min resting adaptation period, participants began a 10-min resting baseline period where they remained quiet. Subsequently, participants were provided with instructions regarding the PASAT and completed a brief practice to ensure they understood the task. Following this, all participants completed the 10-min stress task, as well as a 10-min recovery period (data not reported here). Finally, participants completed several questions regarding the perceived stressfulness and difficulty of the task, as well as their engagement. All questions were scored on a 7-point Likert scale from 0 (not at all) to 6 (extremely). During visit 2, approximately one year later, 40 participants were randomly selected to attend the Birmingham University Imaging Centre for a 1.5 hour visit, during which structural MRI data was collected.

## Acquisition of Brain Imaging Data

Neuroimaging data was obtained using a Philips 3.0T Achieva system with 8-channel head coil. Structural images were acquired using the T1 TFE technique (TR = 8.4ms, TE = 3.8ms, FoV = 232 mm, flip angle = 8° 288 × 288 matrix, 175 slices, voxel size 1 × 1 × 2mm).

**Image Processing**

The Freesurfer 5.3.0 software package (http://surfer.nmr.mgh.harvard.edu) was used to compute volumetric data (Fischl & Dale, 2000). Automated segmentation of the amygdala, hippocampus and insula using this method has been previously described (Morey et al., 2009). Image pre-processing steps included affine registration to Talairach space, skull stripping, intensity bias correction, and spatial normalisation. Data were inspected for outlier volume values. Outlier criteria was defined as any data that exceeded three standard deviations. Additionally, all data was visually inspected. Following visual and statistical inspection, no individuals exceeded our criteria. Subcortical structures were estimated by an automated segmentation and labelling method, based on a combination of structure location, voxel intensity and spatial relationships with proximal subcortical structures (Fischl et al., 2002). Separate left and right hemisphere volumes were summed to provide total volumes for each area of interest. For each subject, an estimate of total intracranial volume (ICV) was obtained by a scaling factor from the Talairach transformation step (Buckner et al., 2004).

**Data Reduction and Statistical Analysis**

Cardiovascular data were averaged for each 10-min period (baseline, stress) and reactivity for each cardiovascular measurement was computed as the difference between baseline average and stress task average. Three participants did not have data for SV, CO and TPR due to poor quality images being obtained with Doppler ultrasound. A repeated measures MANOVA using baseline and task cardiovascular values was performed to confirm the stress task perturbed cardiovascular activity. Pillai's trace was reported as this is considered the

most robust of the multivariate significance tests (Olson, 1976) and alpha level was set at $p < .05$. To determine associations between stressor-evoked cardiovascular reactivity and volumetric grey matter volume of amygdala, hippocampus and insula regions, a series of 2-step hierarchical regressions were run. In all analyses, volumetric data were entered as dependent variables and ICV, age, SES and BMI were entered in step-1 as covariates. Covariates were selected a priori based on previous literature (Gianaros, Kuan, et al., 2017) and as recommended to reduce bias in multiple regression models (Steyerberg, Eijkemans, Harrell, & Habbema, 2001). Cardiovascular variables were entered in step-2, and separate analyses were run for each variable of interest (HR, SBP, DBP, MAP, SV, CO and TPR). Due to the large number of individual regressions, the Benjamini-Hochberg (B-H) method was implemented to reduce the false discovery rate and prevent the likelihood of a type-1 error occurring (Benjamini & Hochberg, 1995). For each brain region, the *p*-values of each regression are ranked and compared to computed B-H critical values which are calculated based on number of tests run with a false discovery rate set at 0.05 (Benjamini & Hochberg, 1995). Significant results occur where *p*-values from the regression are less than B-H critical values. This method conservatively reduces the false discovery rate whilst power is maintained thus effectively correcting for multiple comparisons (Benjamini & Hochberg, 1995; Thissen, Steinberg, & Kuang, 2002).

## Results

### Stressor-evoked Cardiovascular Reactivity and Stress Ratings

Participants rated the PASAT as moderately difficult (4.23 ± 0.92), stressful (4.68 ± 0.99) and engaging (4.13 ± 1.22), implying that the task was successful in inducing acute stress. A repeated measures MANOVA (baseline, stress) including all cardiovascular

parameters revealed a significant multivariate time effect, Pillai's trace = .869, $F(7, 30) = 28.496$, $p < .001$, $\eta_p^2 = .869$, observed power = 100%. Results examined at the univariate level revealed a significant difference in HR, SBP, DBP, SV, CO, MAP and TPR. Post hoc analyses indicated that the stress task significantly increased HR, SBP, DBP, MAP, SV and CO and decreased TPR compared to baseline (see Table 2.2).

**Structural Volumes**

The means for our structural volumes were as follows; hippocampus 7945.31 ± 798.10 $mm^3$, amygdala 3235.51 ± 419.97 $mm^3$, and insula 11276.82 ± 1247.15$mm^3$. The following correlations between brain volumes were found; hippocampus and amygdala volume, $r(38) = .787$, $p < .001$, hippocampus and insula, $r(38) = .629$, $p < .001$, and amygdala and insula, $r(38) = .428$, $p = .005$.

**Stressor-Evoked Cardiovascular Reactivity and Amygdala Grey Matter Volume**

Seven separate regression analyses for each CVR variable were run. Stressor-evoked HR reactivity (β = -.476), CO reactivity (β = -.445), and SBP reactivity (β = -.357), significantly predicted total amygdala grey matter volume, when controlling for ICV age, SES and BMI (see Table 2.3). Following B-H correction, SBP reactivity was no longer significant. Thus, greater increases in HR and CO reactivity predicted lower grey matter volume of the amygdala. No significant associations following B-H correction were evident for SBP, DBP, MAP, SV, or TPR reactivity with amygdala volume. See Figure 2.1 for unadjusted associations between amygdala grey matter volume and all cardiovascular reactivity variables.

**Table 2.2.** Mean (*SD*) values of cardiovascular variables during baseline and stress. Results are reported for repeated measures MANOVA univariate tests.

| | Mean (*SD*) | | *F* | *p* | $\eta_p^2$ |
|---|---|---|---|---|---|
| | Baseline | Stress | | | |
| Heart rate (bpm) | 74.75 (12.63) | 91.31 (14.57) | 157.47 | <.001 | .814 |
| Systolic blood pressure (mmHg) | 109.03 (9.59) | 121.98 (11.65) | 163.86 | <.001 | .820 |
| Diastolic blood pressure (mmHg) | 67.78 (6.45) | 76.99 (8.61) | 107.50 | <.001 | .749 |
| Mean arterial pressure (mmHg) | 81.69 (7.50) | 93.06 (9.38) | 173.50 | <.001 | .828 |
| Stroke volume (ml) | 71.87 (10.15) | 76.23 (11.86) | 22.97 | <.001 | .390 |
| Cardiac output (L/min) | 5.20 (0.89) | 6.94 (1.42) | 119.25 | <.001 | .768 |
| Total peripheral resistance (dyne-s/$cm^{-5}$) | 1290.89 (189.15) | 1117.05 (198.73) | 50.06 | <.001 | .582 |

**Table 2.3.** Regression coefficients and *p* values for regressions between stressor-evoked cardiovascular reactivity and total amygdala, hippocampus and insula volume controlling for intracranial volume, age, socio-economic status and body mass index.

| | Amygdala | | | Hippocampus | | | Insula | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\Delta R^2$ | β | *p* | $\Delta R^2$ | β | *p* | $\Delta R^2$ | β | *p* |
| ΔHR (bpm) | .202 | -.476 | .002* | .120 | -.367 | .008* | .004 | -.067 | .597 |
| ΔSBP (mmHg) | .106 | -.357 | .031 | .101 | -.350 | .016* | .000 | -.006 | .964 |
| ΔDBP (mmHg) | .016 | -.129 | .418 | .004 | -.067 | .638 | .012 | .111 | .363 |
| ΔMAP (mmHg) | .056 | -.248 | .122 | .042 | -.214 | .132 | .000 | .020 | .874 |
| ΔSV (ml) | .008 | -.095 | .584 | .019 | -.145 | .337 | .002 | .052 | .685 |
| ΔCO (L/min) | .183 | -.445 | .006* | .131 | -.376 | .008* | .000 | -.018 | .889 |
| ΔTPR (dyne-s/cm$^{-5}$) | .098 | .320 | .051 | .076 | .283 | .051 | .001 | .031 | .805 |

*Note*: ΔHR: Heart Rate reactivity; ΔSBP: Systolic Blood Pressure Reactivity; ΔDBP: Diastolic Blood Pressure Reactivity; ΔSV: Stroke Volume Reactivity; ΔCO: Cardiac Output Reactivity; ΔMAP: Mean Arterial Pressure Reactivity; ΔTPR: Total Peripheral Resistance Reactivity. Separate regressions were run for each cardiovascular variable. **Remains significant after adjusting for Benjamini-Hochberg correction FDR = 0.05.*

### Stressor-Evoked Cardiovascular Reactivity and Hippocampus Grey Matter Volume

Separate regression analyses found HR reactivity (β = -.367), CO reactivity (β = -.376) and SBP reactivity (β = -.350) significantly predicted total hippocampus grey matter volume when controlling for ICV, age, SES and BMI (see Table 2.3). Significant results remained following B-H correction. Specifically, greater increases in HR, SBP, and CO reactivity predicted lower grey matter volume of the hippocampus. No significant associations were evident with DBP, MAP, SV, or TPR reactivity and hippocampus volume. See Figure 2.2 for unadjusted associations between hippocampus grey matter volume and all cardiovascular reactivity variables.

### Stressor-Evoked Cardiovascular Reactivity and Insula Grey Matter Volume

Seven separate regression analyses controlling for ICV, age, SES and BMI found no significant relationships between CVR parameters and insula grey matter volume (see Figure 2.3 for unadjusted associations between insula grey matter volume and all cardiovascular reactivity values). In addition, B-H correction did not alter the results (see Table 2.3).

## Discussion

This study examined the relationship between stressor-evoked cardiovascular reactivity and amygdala and hippocampal volume. In line with our hypotheses, increased stressor-evoked cardiovascular reactivity was associated with reduced amygdala and hippocampal volume, but was not associated with insula grey matter volume. Specifically, greater HR, CO, and SBP reactivity were associated with reduced hippocampus and HR and CO reactivity were associated with reduced amygdala volume. There were no associations between DBP, MAP, SV, nor TPR and amygdala or hippocampus grey matter volume. The finding that cardiovascular reactivity does not relate to insula volume, an area implicated in

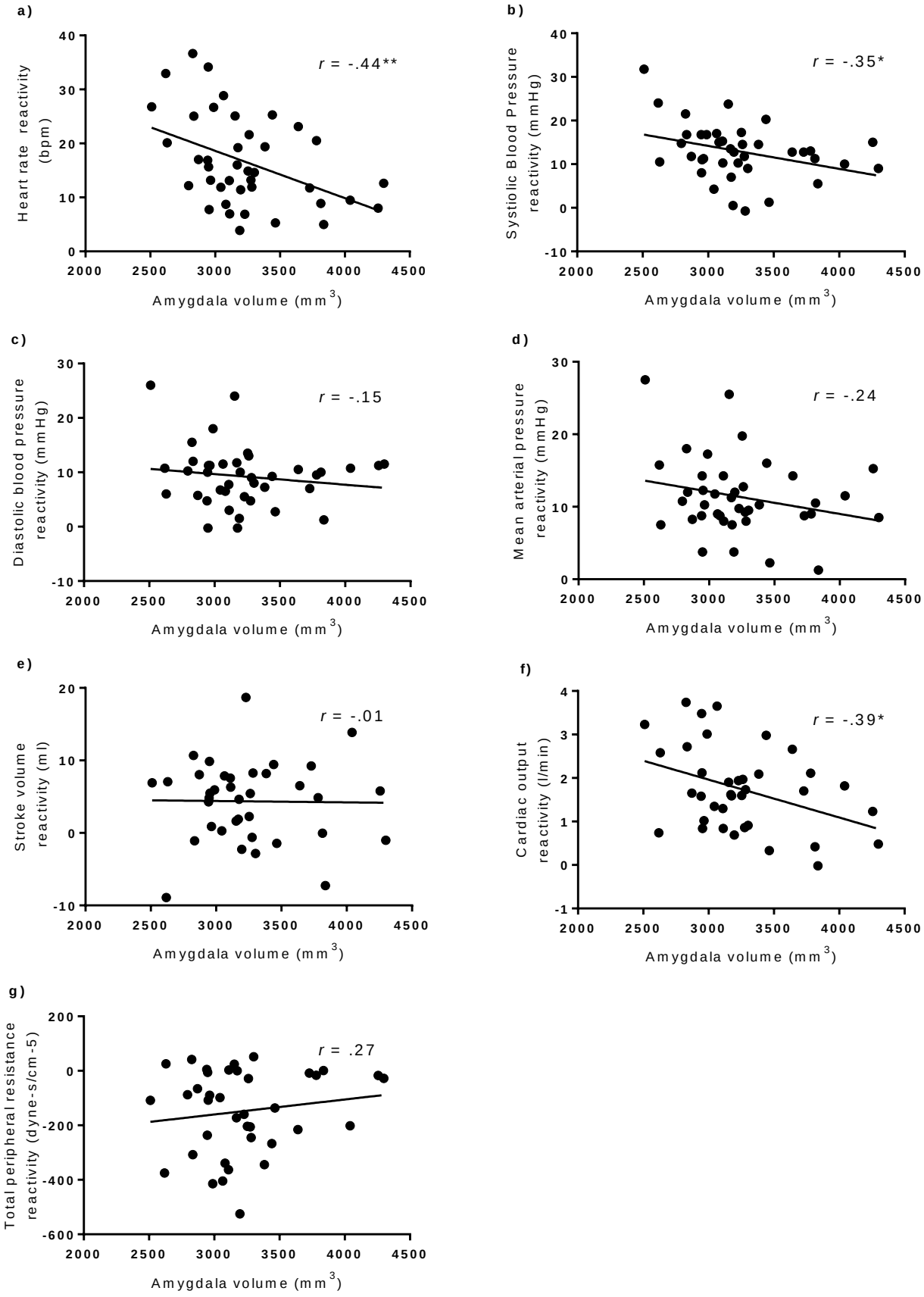


**Figure 2.1.** Scatterplots showing unadjusted amygdala volume plotted against a) heart rate reactivity b) systolic blood pressure reactivity c) diastolic blood pressure reactivity d) mean arterial pressure reactivity e) stroke volume reactivity f) cardiac output reactivity g) total peripheral resistance reactivity. *$p < .05$. **$p < .01$.

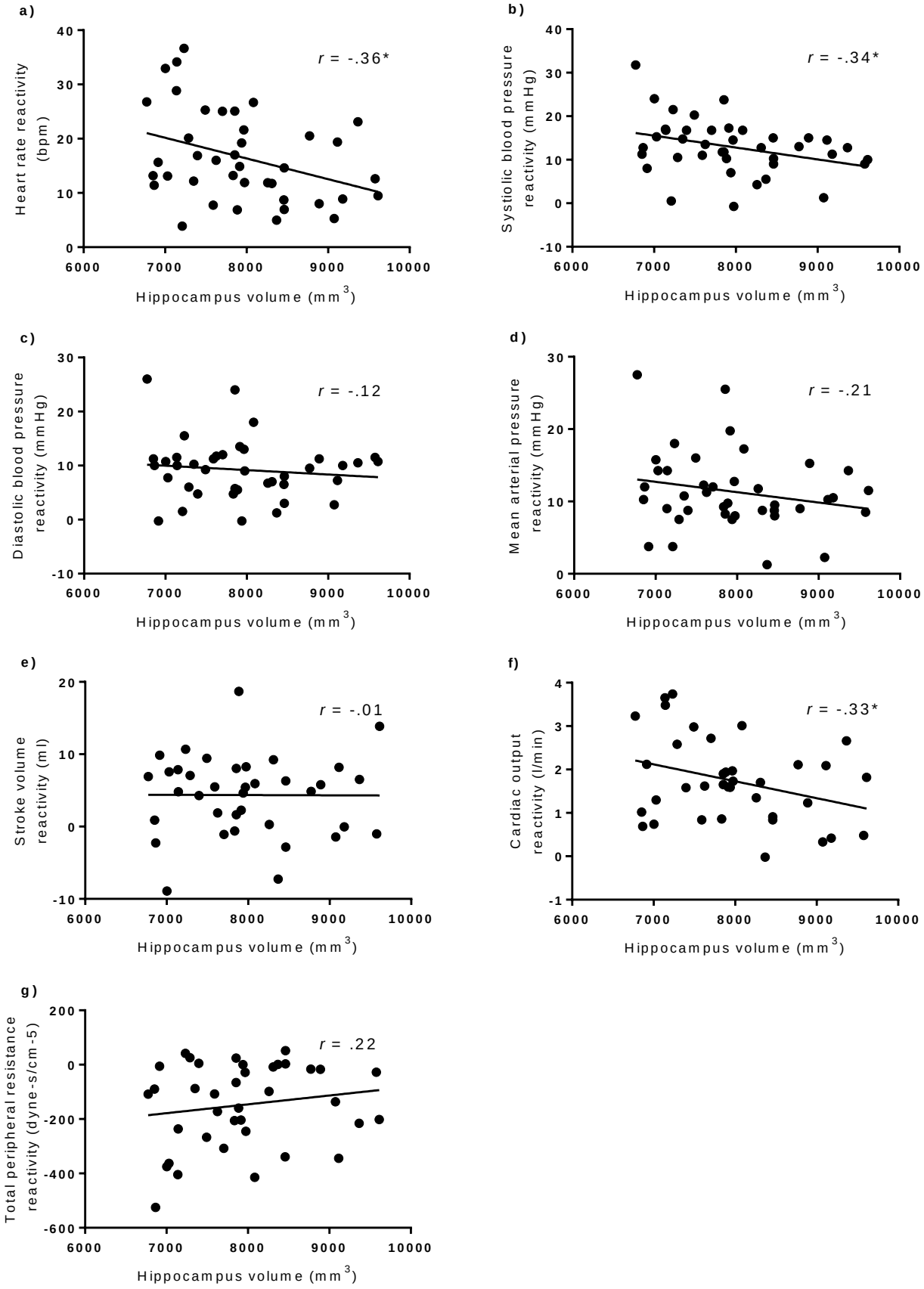


**Figure 2.2.** Scatterplots showing unadjusted hippocampus volume plotted against a) heart rate reactivity b) systolic blood pressure reactivity c) diastolic blood pressure reactivity d) mean arterial pressure reactivity e) stroke volume reactivity f) cardiac output reactivity g) total peripheral resistance reactivity. *$p < .05$.

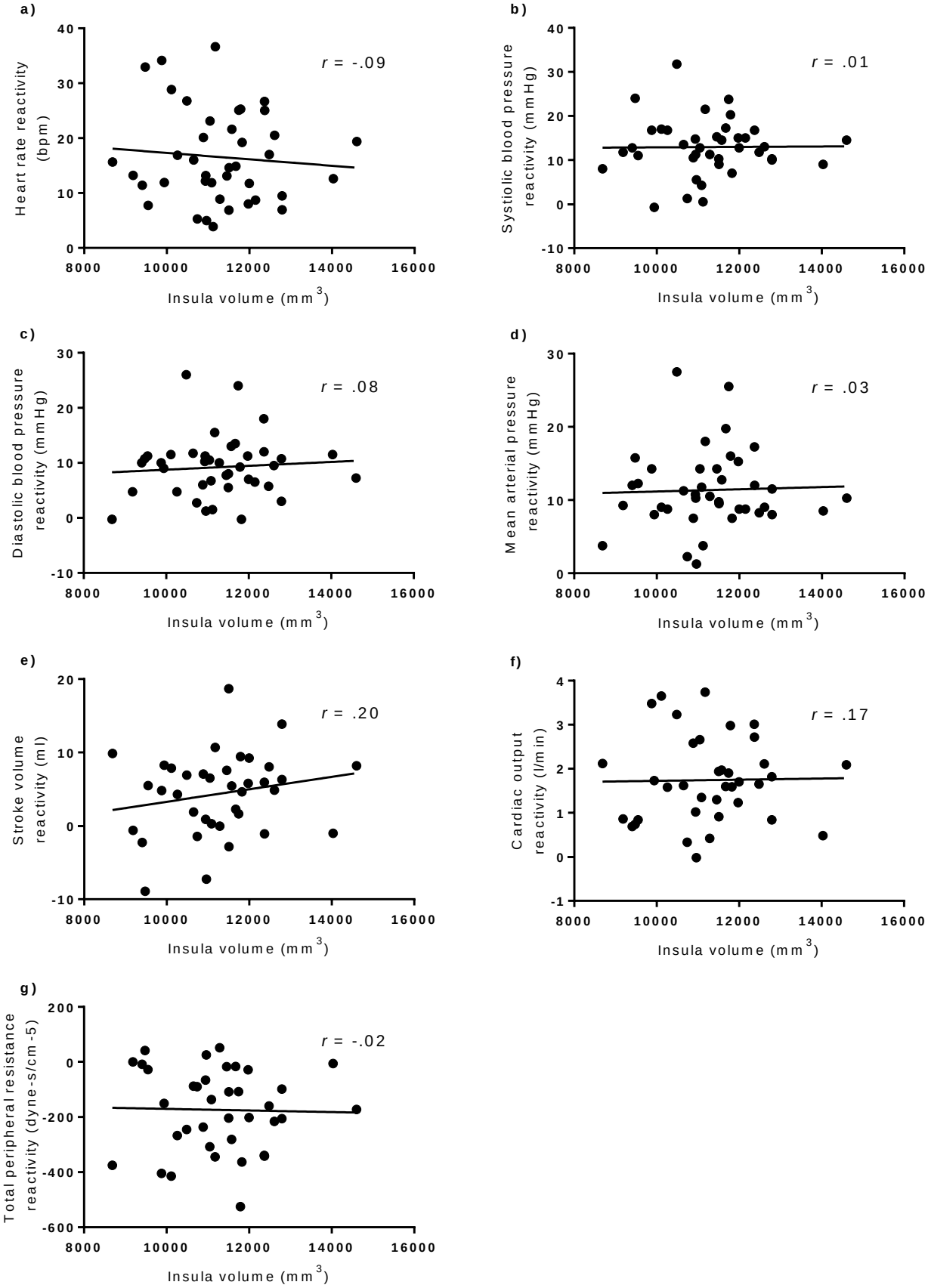


**Figure 2.3.** Scatterplots showing unadjusted insula volume plotted against a) heart rate reactivity b) systolic blood pressure reactivity c) diastolic blood pressure reactivity d) mean arterial pressure reactivity e) stroke volume reactivity f) cardiac output reactivity g) total peripheral resistance reactivity.

visceral control (Beissner et al., 2013; Oppenheimer & Cechetto, 2016), provides evidence that our observed relationship between cardiovascular reactivity and brain morphology may be more closely—although not exclusively—linked to medial temporal areas that are thought to influence peripheral stress physiology; namely, the amygdala and hippocampus. Whole-brain computational approaches that integrate other aspects of brain tissue morphology and cortical and subcortical areas are needed to fully explore this possibility.

In contrast with the only other human study explicitly examining the associations between stressor-evoked cardiovascular reactivity with brain morphology, where MAP was associated with reduced amygdala and hippocampal grey matter volume (Gianaros et al., 2008), we found no associations between brain morphology and MAP reactivity. Several reasons could underlie the discrepancies between our MAP results and Gianaros et al. (2008). First, we measured cardiovascular parameters in a laboratory setting whereas Gianaros et al. (2008) measured MAP while concurrently completing the fMRI protocol in the scanner. Consequently, participants were in a different postural position whilst completing the stress task and these postural alterations could result in altered cardiovascular responses to the stressor (Sherwood & Turner, 1993; Turner, 1994).

Second, although both studies employed validated stressors, the stress tasks were different. Different stress tasks are known to produce distinctive underlying hemodynamic responses which ultimately lead to blood pressure changes (Kasprowicz, Manuck, Malkoff, & Krantz, 1990). The present study sample experienced a greater increase in MAP compared to Gianaros et al. (2008), suggesting the different tasks may account for the discrepancy in the results. Finally, while both studies computed amygdala and hippocampus volumes with validated automated segmentation methods (Dale, Fischl, & Sereno, 1999; Whitwell, 2009), the software used for automated segmentation differed across studies and could possibly

underlie the discordance across the studies (Grimm et al., 2015). The current study used FreeSurfer and the Gianaros et al. (2008) study used voxel-based morphometry.

An interesting observation is that HR and CO reactivity (SBP reactivity prior to B-H correction), but not DBP, TPR, SV, or MAP reactivity relate to amygdala and hippocampus volumes. While the former group of cardiovascular variables are suggested to be cardiac driven, and the latter group more vascular driven (Kasprowicz et al., 1990; Llabre, Klein, Saab, McCalla, & Schneiderman, 1998; Sherwood, Dolan, & Light, 1990), arguably it could be that more cardiac driven responses to psychological stress, rather than vascular responses, may more strongly be associated with reduced volumes of the amygdala and hippocampus. It is possible that while Gianaros et al. (2008) found associations with MAP, this is a cardiovascular end point, and our study's more extensive cardiovascular parameters highlight that cardiac variables may be underlying this association.

The hippocampus and amygdala are rich with glucocorticoid receptors which are low-affinity receptors and are thus bound during periods of high glucocorticoid levels such as during acute stress (Jacobson & Sapolsky, 1991; Wang et al., 2014). Although the present study did not measure markers of the HPA-axis, greater adrenocorticotrophic hormone and cortisol responses are associated with greater stressor-evoked cardiovascular reactivity (Al'Absi 1997) and cardiovascular reactivity to acute psychological stress has been shown to be greatly enhanced by hypothalamic-pituitary-adrenal (HPA) activity (Herd, 1986; Walker, Best, Shackleton, Padfield, & Edwards, 1996). It may be that individuals with atrophied volume of medial temporal lobe regions, such as the hippocampus and amygdala, exhibit an impaired negative feedback of the HPA-axis, which results in inefficient restraint over cardiovascular or related responses to stress (Boyle et al., 2005; Furay, Bruestle, & Herman, 2008). Human studies supporting this hypothesis include individuals with Cushing's disease

(Starkman, Gebarski, Berent, & Schteingart, 1992), where individuals produce excessive levels of glucocorticoids, and show reduced medial temporal lobe volumes, particularly in the hippocampus. Following surgical correction for the hypercortisolemia, the volumetric reductions are largely remediated (Starkman et al., 1999). Owing to the cross-sectional design of the present study and lack of HPA measurements, we are unable to draw inferences about causal associations, temporal ordering of associations, or the role of glucocorticoids in linking medial temporal lobe volumes with stressor-evoked cardiovascular reactivity. Accordingly, these suggestions warrant further study.

Secondly, the reactivity hypothesis states that greater individual stressor-evoked cardiovascular reactivity is associated with increases in future blood pressure status (Carroll, Ginty, Der, et al., 2012). Evidence has shown that higher blood pressure levels are associated with brain volume reductions in later life, particularly in the hippocampus (Beauchet et al., 2013; Jennings et al., 2012). Thus, it is plausible that that greater stressor-evoked cardiovascular reactivity could have neurodegenerative effects over the lifespan, specifically causing atrophy of the hippocampus. The suggested explanation underlying the effects of hypertension on brain volume is that hypertension causes atherosclerosis, resulting in blood vessel diameter changes, and consequently reduced perfusion to capillary beds in the brain (Beauchet et al., 2013; Dai et al., 2008). As a result of this hypo-perfusion, regions including the frontal lobe and hippocampus are at increased risk for atrophy (Cohen, 2007). However, this perspective has been challenged by recent findings showing that neural alterations precede prospective increases in blood pressure, leaving unresolved the question of whether blood pressure is in fact causal to brain alterations (Jennings et al., 2017). Notwithstanding, our study population was comprised of a very young and healthy group of women at low cardiovascular disease risk. Therefore, it seems unlikely that brain volume reductions are a

consequence of hypertension or cardiovascular disease burden. Rather, it could be suggested that brain volume reductions might predispose individuals toward stress sensitivity and greater reactivity (Gilbertson et al., 2002).

To elaborate, there is evidence to suggest that reduced volumes of the hippocampus and amygdala are heritable and may predispose individuals to increased stress-related responsiveness (Gianaros et al., 2008; Meyer-Lindenberg et al., 2006; Morey et al., 2009; Pezawas et al., 2005). For example, Gilberston et al. (2002) implementing a monozygotic twin design found stronger evidence for heritability influencing hippocampal volumes than stress exposure in twins where one of the twins had combat exposed PTSD. Animal work has shown that hippocampal size was dependent on genetic heritability (54%), but not different postnatal conditions. Importantly, monkeys with small hippocampal volumes experienced relatively exaggerated cortisol responses to social stress (Lyons et al., 2001). This indicates that reduced hippocampal volume increases the vulnerability of exhibiting exaggerated physiological stress responses. Complementing the above studies, in healthy adults, classified into high and low perceived stress groups based on the perceived stress questionnaire, groups differed in their hippocampal volumes. The high perceived stress group exhibited smaller hippocampal volumes compared to the low stress group, and over a 5-year period, neither the high or low stress groups displayed changes in perceived stress or hippocampal volume (Lindgren et al., 2016). It was therefore concluded that because no changes were found over the 5 years, smaller hippocampal volumes may be a vulnerability factor, contributing to experiencing exaggerated stress reactions (Lindgren et al., 2016). Together, the above studies indicate that smaller hippocampal volumes appear more likely to be a result of dispositional, and possibly heritable factors, rather than a consequence of continued exposure to stress. Indeed, individuals with smaller hippocampal volumes may be at risk for experiencing

chronic psychological and physiological distress (Van Rooij et al., 2015). However, it should be acknowledged that the current study has a small size, therefore, our results should be replicated in a larger sample.

In agreement with evidence implicating reduced volumes of the hippocampus as a vulnerability to developing stress related hyper responsivity, evidence also implicates the amygdala as an important structure leading to exaggerated stress response. Animal work reveals that mice strains with small basolateral volumes of the amygdala express augmented fear and cortisol responses compared to those with larger amygdala volumes (Yang et al., 2008). Moreover, mice strains with small basolateral amygdala display an exaggerated cortisol response when subjected to a stress condition, which was not apparent in the medium or large amygdala mice strains (Yang et al., 2008). Further evidence demonstrates that individuals carrying genetic variants with increased risk of developing stress-related psychopathology, exhibit pronounced amygdala volumetric reductions (Meyer-Lindenberg et al., 2006; Pezawas et al., 2005). Importantly, hyper-responsive amygdala activity during fear processing was coupled with reduced amygdala volume, suggesting that reduced amygdala volume may directly couple with hyper-responsive amygdala activity (Meyer-Lindenberg et al., 2006; Pezawas et al., 2005). This could potentially explain the relationship relating structural volume of the amygdala to stressor-evoked physiological responses (Pezawas et al., 2005, Gianaros et al., 2008, Meyer-Lindenberg et al., 2006). Thus, it may be that reduced grey matter volumes of the amygdala and hippocampus, could be partly heritable indicators of stress-related phenotypes.

The current study is not without limitations. First, due to the cross-sectional nature of the study, cause and effect and temporal ordering cannot be determined. Second, the current study consisted of a small sample of only females. However, our analysis which included B-H

correction for multiple comparison which is a conservative method, in addition to reporting null findings with the insula as a comparison brain volume arguably offers more confidence in the results. Despite this, the replication of this study's findings in a larger sample including males is essential. Third, brain morphology is known to undergo changes over the course of a year (Cahn et al., 2002), therefore it must be acknowledged that the reported results may be less reliable than if the stress testing and structural scan occurred over a shorter re-test period. However, research has shown cardiovascular reactivity is relatively stable over a 1-month period (Kamarck, Jennings, Stewart, & Eddy, 1993) as well as over an 18-year period (Hassellund, Flaa, Sandvik, Kjeldsen, & Rostrup, 2010). Fourth, the test re-test reliability of amygdala volume using automated segmentation in Freeseurfer has been found to be low (intraclass correlation coefficient 0.6; Morey et al., 2010). In contrast, cardiovascular responses to acute psychological stress are relatively stable across repeated testing sessions and with different tasks (Ginty et al., 2013). To overcome reliability issues inherent with small data sets, replication of the our novel study results should be replicated in a larger sample. Lastly, other potential mediators of grey matter volume reductions have been implicated such as inflammation (Marsland, Gianaros, Abrarnowitch, Manuck, & Hariri, 2008; O'Donovan et al., 2015). Future research should include measures of inflammatory markers to explore the role that inflammation may have on the relationship between stress and limbic morphology.

In conclusion, the current study demonstrates that reduced volumes of the amygdala and hippocampus are associated with greater stressor-evoked cardiovascular reactivity. Future longitudinal studies are needed to delineate the causal bases of associations between brain structure and stressor-evoked cardiovascular reactivity, which is a known correlate and predictor of future cardiovascular risk.

# CHAPTER 3

## PSYCHOLOGICAL AND PHYSIOLOGICAL RESPONSES TO ACUTE MENTAL STRESS FOLLOWING ACUTE HIGH INTENSITY EXERCISE

This chapter is currently under review:

Trotman, G. P., Veldhuijzen van Zanten, J. J. C. S., Ginty, A. T., & Williams, S. E. (under review). Psychological and physiological responses to acute mental stress following acute high intensity exercise. *Psychology of Sport and Exercise*.

## Chapter 3 Preface

Chapter 2 revealed that amygdala and hippocampus brain morphology were associated with cardiovascular reactivity during acute psychological stress. These results highlight that biological factors are related to individual differences in cardiovascular stress reactivity and could be dispositional factors that increase the vulnerability for experiencing greater stress. Given that responses to stress are associated with brain regions involved with threat perception and the processing of environmental relevance, it is important to explore whether the responses to acute psychological stress can be altered using an acute intervention. If individuals with a dispositional vulnerability to experience exaggerated stress (i.e., individuals with reduced amygdala and hippocampus volumes) could engage in an acute intervention which acutely attenuates stress responses, this would be an important adaptive intervention for individuals with greater susceptibility to experience the negative consequences of exaggerated stress reactivity. A behaviour which is suggested to positively influence physical and mental health is exercise. However, the extent to which acute exercise can alter the responses to stress is not currently clear. Chapter 3 therefore used an acute high intensity exercise paradigm prior to acute psychological stress, to examine whether the stress responses to acute stress can be attenuated. When completing an intervention to change the responses to stress, it is important to consider the both the physiological and the psychological responses given both of their importance for health and well-being. Consequently, cardiovascular and psychological responses during stress were measured to examine whether an acute high intensity exercise paradigm influences the psychological responses to stress.

## Abstract

Acute exercise prior to stress attenuates subsequent stressor-evoked blood pressure responses. However, little is known about how acute exercise affects subsequent psychological responses to stress. The study aimed to examine the effects of an acute bout of exercise on the psychological and physiological responses to acute psychological stress. Forty healthy participants (20 male; mean age = 19.95 ± 1.93 years) completed three laboratory sessions on separate days. Visit one: a sub-maximal exercise test was completed to calculate estimated $VO_{2max}$. Visit two (stress only): 10-min resting baseline followed by a standardized psychological stress task (multisource interference task). Visit 3 (stress following exercise): 10-min cycle at 70% $VO_{2max}$ and 20-min recovery, followed by resting baseline and MSIT. Visit two and three were counterbalanced. Psychological measures including, primary and secondary appraisals, cognitive and somatic anxiety, and mood states were measured immediately following stress. Heart rate and blood pressure were analyzed during pre-stress baseline and stress. As expected, exercise reduced systolic blood pressure responses to stress ($p$ = .002). Compared to stress only, during stress following exercise, lower somatic anxiety ($p$ =.015) but greater perceived threat ($p$ =.010), stress ($p$ =.024), and difficulty ($p$ =.001), and greater tension ($p$ =.012), depression ($p$ =.026), fatigue ($p$ =.003), confusion ($p$ =.033), and lower vigour ($p$ =.022) were reported. Acute high intensity exercise prior to acute psychological stress lowers somatic anxiety and systolic blood pressure responses, yet has a detrimental effect on primary appraisals and mood states experienced during stress.



**Keywords:** Acute Psychological Stress; Exercise; Anxiety; Mood; Stress Appraisal; Cardiovascular Reactivity.

## Introduction

Acute psychological stress can elicit negative psychological responses (e.g., increases in anxiety and negative mood) (Hilmert & Kvasnicka, 2010) which have been associated with negative health outcomes (Kivimäki & Steptoe, 2018. For example, negative moods and emotions (e.g., anxiety), and appraisals of stress are associated with psychological disorders including anxiety and depression (Charles et al., 2013), and are implicated as triggers for cardiovascular events (Edmondson, Newman, Whang, & Davidson, 2013; Kivimäki & Steptoe, 2018). As such, it is important to explore ways to reduce these negative psychological responses to acute psychological stress. While it is known that exercise training and physical activity are beneficial for physical and mental health (Basso & Suzuki, 2017; Penedo & Dahn, 2005; White et al., 2017), and have been associated with lower levels of perceived stress (Dogra et al., 2018; Stults-Kolehmainen & Sinha, 2014), less attention has been given to the potential of using acute bouts of exercise as a stress regulation strategy prior to experiencing stressful events.

Interestingly, literature has consistently demonstrated that an acute bout of exercise prior to stress exposure can attenuate the stressor-evoked blood pressure response (Brownley et al., 2003; Hamer et al., 2006). Meta-analyses have revealed the largest effect on blood pressure reactivity reductions are seen with exercise intensities greater than 60% $VO2_{max}$ that lasted for at least 20 minutes (Hamer et al., 2006). As such, high intensity exercise is thought be the most beneficial for reducing blood pressure responses to stress (Alderman et al., 2007; Hamer et al., 2006). However, there is less evidence examining the effects of high intensity acute exercise on the subsequent psychological responses to acute psychological stress, and the studies that have examined this report equivocal findings.

Exercise is thought to be beneficial for psychological health and well-being, with substantial literature demonstrating that acute exercise can reduce negative emotions such as state anxiety (Ensari, Greenlee, Motl, & Petruzzello, 2015) and enhance resting positive moods (Yeung, 1996). For example, Smith, O'Connor, Crabbe, and Dishman (2002) demonstrated 25-mins of cycling lead to reductions in state anxiety. Similarly, Szabo (2003) demonstrated that 20-mins of jogging lead to reductions in mood disturbance. Therefore, it could be suggested that acute exercise prior to stress exposure would have similar beneficial effects on these feelings and emotions experienced during the stress. However, the limited research examining the effects of acute exercise on these responses experienced in response to stress have revealed mixed findings. Specifically, research has demonstrated that exercise prior to stress can have no effect (McGowan et al., 1985; Roy & Steptoe, 1991), increase negative emotions (Duda et al., 1988; Steptoe et al., 1993), and result in fewer negative emotions (Benvenutti et al., 2017; Roth, 1989) experienced during stress.

Similar to the blood pressure responses, the effect of exercise on the psychological responses to stress might be influenced by the intensity of the exercise, with high intensity exercise provoking a negative effect on psychological responses to stress (Duda et al., 1988; Steptoe et al., 1993). For example, Steptoe et al. (1993) demonstrated greater levels of tension-anxiety following 20-mins of cycling at $VO2_{max.}$ Therefore, whilst high intensity exercise may attenuate blood pressure responses to stress, this adaptive response may not be extended to the psychological responses, and may even be detrimental in nature. To explore this hypothesis, a comprehensive examination of the psychological responses to stress following exercise is needed.

It is thought that psychological stress responses such as anxiety and mood, are influenced by how an individual appraises a stressful situation (Gianaros & Jennings, 2018;

Lazarus, 1999). Stress appraisals can be categorised into primary appraisals, where an individual interprets the personal relevance of a situation (e.g., threat, demand, difficulty, stressfulness), as well as secondary appraisals, where an individual evaluates their available resources (e.g., ability to cope, perceived control) (Lazarus & Folkman, 1984). It is proposed that these appraisals are continually updated by contextual and situational factors (Gianaros & Jennings, 2018; Lazarus & Folkman, 1984). Performing high intensity exercise will use up physical and mental resources. It can be proposed that depleted resources are likely to negatively influence both primary and secondary appraisals (i.e., the individual is likely to perceive the situation as more stressful, threatening, demanding, and feel they have fewer available resources to cope). Because lower resources and greater task demands are associated with more negative emotions during stress (Jones, Meijen, McCarthy, & Sheffield, 2009), it can subsequently be proposed that these alterations in primary and secondary appraisals caused by exercise could underlie the resulting mood and anxiety responses. However, research has yet to examine the effects of exercise on subsequent appraisals of acute psychological stress exposure.

Anxiety is one of the most prevalent mental disorders and is associated with a high disease burden (Bandelow & Michaelis, 2015) and psychological stress is one factor known to contribute to its development (Baum & Posluszny, 1999; Kahn & Khan, 2017). Despite the importance of understanding strategies to alleviate anxiety symptoms, studies examining the effect of acute exercise on anxiety have typically used general anxiety assessments, including subscales from mood states (e.g., the profile of mood states questionnaire) (Steptoe et al., 1993). However it is important to highlight these anxiety assessments do not separately assess anxiety's varying dimensions; that is its cognitive and somatic components, as well as the perceived interpretation of these anxiety symptoms (Jones & Swain, 1992; Steptoe &

Kearsley, 1990). This distinction is important when investigating anxiety and exercise, particularly when exercising at higher intensities. Indeed, the validity of state anxiety questionnaires may be compromised with questionnaires tapping somatic activation and energetic arousal rather than anxiety per se (Ekkekakis, Hall, & Petruzzello, 1999; Rejeski, Hardy, & Shaw, 1991). As such, implementing a more detailed assessment of anxiety, which differentiates between cognitive (i.e., negative thoughts and concerns) and somatic (i.e., physiological arousal) symptoms could help explain some of the variation of previous studies using unidimensional measures of anxiety.

To our knowledge, only two studies have examined the effect of exercise on cognitive and somatic anxiety in response to stress. In one study, the effect of yoga, a low intensity exercise, on cognitive and somatic intensity and perceived interpretation during stress revealed no effects (Benvenutti et al., 2017). In contrast, Duda et al. (1988) found greater somatic anxiety during stress following high intensity exercise (70% of $VO2_{max}$), but the exercise had no effect on cognitive anxiety. Although perceived anxiety interpretation was not assessed, the findings highlight the importance of separately assessing cognitive and somatic anxiety following higher intensity exercise. Furthermore, the finding supports the idea that preceding high intensity exercise is likely to be detrimental to some psychological responses. Understanding how anxiety is interpreted during stress is of importance, as evidence suggests the interpretation is potentially more influential on performance than anxiety intensity (Jones et al., 1993). It could be that following higher intensity exercise, with greater somatic anxiety, individuals may perceive this as more debilitating towards how they will perform during stress. Exploring whether exercise can induce more facilitative or debilitative anxiety interpretations is important to understand the effects of acute exercise for stress regulation.

Using a within-subject design, the present study explored the detailed psychological responses to acute psychological stress following high intensity aerobic exercise. Seventy percent $VO_{2max}$ exercise was selected as this is classified as high intensity exercise (Garber et al., 2011), which is known to induce the greatest attenuation in blood pressure during subsequent acute psychological stress (Alderman et al., 2007; Hamer et al., 2006). Psychological variables including primary (e.g., perceived threat) and secondary (perceived coping) stress appraisals, categorised based on Lazarus and Folkman's transactional model of stress and coping (Lazarus & Folkman, 1984), cognitive and somatic anxiety intensity and symptom interpretation, and mood, were assessed under two different conditions: stress only and stress following an acute bout of high intensity exercise (70% $VO_{2max}$).

It was hypothesised that compared to a stress only condition, during the stress task following high intensity exercise, participants would experience attenuated blood pressure responses, but that they would also experience 1) greater perceived demands, threat, difficulty, and stressfulness of the task (i.e., primary appraisals), and lower ability to cope and perceived control of the task (i.e., secondary appraisals), 2) greater negative mood responses, 3) greater somatic anxiety and more debilitative symptom interpretation, 4) no effect on cognitive anxiety or symptom interpretation

## Method

### Participants

Based on previous work examining the effects of high intensity acute exercise on blood pressure reactivity, a medium to large effect size was expected (Hamer et al., 2006). Power analyses (alpha= .05, power .90, medium effect size) using G*Power revealed that a sample size of 36 was required (Faul, Erdfelder, Lang, & Buchner, 2007). Forty (20 women, 20 men; Mean age = 19.95, SD = 1.93 years, see Table 3.1 for demographics) healthy

non-smokers with no history of immune, cardiovascular, metabolic, or kidney disease participated in the study, to allow for potential dropout. All were asked to abstain from alcohol and vigorous exercise for 12hr, caffeine for 2hr, and food and drink other than water for 1hr before testing. All participants provided written informed consent prior to testing, and were provided with either undergraduate course credit or monetary compensation (£20) upon completion of the study. The study was approved by the University Ethics Committee.

**Table 3.1.** Mean (SD) participant demographics

| | Male | Female |
|---|---|---|
| Age (years) | 19.95 (2.35) | 19.95 (1.47) |
| Height (cm) | 180.25 (7.98) | 166.48 (7.33) |
| Weight (kg) | 73.58 (10.93) | 59.01 (6.16) |
| BMI ($kg/m^2$) | 22.65 (3.01) | 21.32 (2.09) |
| $VO_{2max}$ (ml/kg/min) | 44.96 (9.67) | 37.79 (9.00) |
| Ethnicity | | |
| White | 15 (75%) | 16 (84.1%) |
| Black | 0 (0%) | 1 (5.3%) |
| Other | 5 (25%) | 2 (10.6%) |

*Note:* body mass index (BMI)

## Physiological Measures

**Cardiovascular assessment.** Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained using an oscillatory blood pressure monitor (Omron HEM-705CP), with a cuff attached to the participant's non-dominant upper arm. Heart rate (HR) was recorded continuously with the ambulatory monitoring system, VU-AMS5fs (TD-FPP, Vrije Universiteit, Amsterdam, the Netherlands) which has been shown to reliably record valid cardiovascular measures (de Geus, Willemsen, Klaver, & Vandoornen, 1995). The electrocardiogram (ECG) was analyzed offline, with automated inter-beat interval time series

detection using the VU-DAMS software. In addition, following manual inspection, artefacts were removed and corrected where necessary.

**Metabolic assessment.** Breath-by-breath measurements of oxygen consumption ($VO_2$; $ml \cdot min^{-1}$) were performed using an online automated gas analysis system (Oxycon Pro, Jaeger, Hoechberg, Germany). Oxygen and carbon dioxide concentrations were simultaneously measured via a sampling line and all data were recorded and stored on a computer and analyzed offline. The Oxycon system has been shown to reliably measure metabolic activity whilst participants remain seated on a cycle ergometer at rest and during exercise (Foss & Hallén, 2005; Macfarlane & Wong, 2012).

**Psychological Measures**

Cognitive appraisals were categorized based on the transactional model of stress and coping (Lazarus & Folkman, 1984). That is, primary appraisals represent the evaluation of personal relevance or threat to well-being and secondary appraisals represent the perceived controllability and coping evaluations within a given situation.

**Primary stress appraisals.** Three separate items assessed how demanding, stressful, and difficult (e.g., "how difficult did you find the task") participants found the task to be. Ratings were made on a 7-point scale from 0 (not at all) to 6 (extremely). Perceived threat was assessed with 3-items (e.g., "I felt threatened by the situation") ranging from 0 (not at all true) to 6 (very true), which were averaged into a single threat score (McGregor & Elliot, 2002; Trotman, Williams, Quinton, & Veldhuijzen Van Zanten, 2018).

**Secondary stress appraisals.** Perceived control (e.g., how much control did you feel you had), and ability to cope (e.g., "to what extent were you be able to cope") with the task demands were each assessed with two items. Responses were made on a 7-point scale ranging

from 0 (no/not at all) to 6 (total/completely), as used in previous research (Trotman et al., 2018).

**Mood state.** The modified profile of mood states (POMS) (McNair et al., 1971) assessed tension (e.g., nervous), depression (e.g., unhappy), anger (e.g., grouchy), fatigue (e.g., exhausted), confusion (e.g., muddled), and vigour (e.g., energetic) using 32 items. Items were rated on a 5-point scale between 0 (not at all) and 4 (extremely). The POMS provides reliable and valid measures of mood states (Terry, Lane, & Fogarty, 2003), with Cronbach alphas for the six subscales reported from .76 to .95 (Studts, 1995) and has been used extensively to assess exercise and stress related affective states (Szabo, 2003; Terry et al., 2003).

**Anxiety.** The Immediate Anxiety Measures Scale (IAMS) (Thomas, Hanton, & Jones, 2002) assessed the intensity of both cognitive and somatic anxiety (1 = not at all to 7 = extremely) as well as perceived interpretation of each of these symptoms experienced (-3 = very debilitative/negative to +3 = very facilitative/positive). Participants first completed the intensity rating followed by the perceived interpretation for cognitive anxiety, before moving onto somatic anxiety. Confidence was not included in the current study. The IAMS produces valid and reliable scores of cognitive and somatic anxiety, particularly in close proximity to an event (Thomas et al., 2002) and is used frequently to assess stress specific anxiety (Trotman et al., 2018; Williams, Veldhuijzen van Zanten, Trotman, Quinton, & Ginty, 2017).

**Task engagement.** Participants rated how engaged they were in the task on a 7-point Likert scale between 0 (not at all) and 6 (extremely), as commonly used in stress literature (Ginty et al., 2013).

**Acute Psychological Stress Task**

The multisource interference task (MSIT) (Sheu, Jennings, & Gianaros, 2012) is a standardized acute psychological stress task, that has been shown to reliably elicit physiological and psychological stress responses and has demonstrated moderate to strong test-retest reliability (Gianaros, Onyewuenyi, Sheu, Christie, & Critchley, 2012; Gianaros, Sheu, et al., 2017; Ginty et al., 2013). A within study design demonstrated individual differences in stressor-evoked cardiovascular responses on the MSIT have been shown to be correlated with individual differences in stressor-evoked cardiovascular responses on a standard mental arithmetic task (i.e., Paced Auditory Serial Addition task) (Ginty et al., 2013).

Briefly, three numbers ranging between 0 and 3 are presented on a computer screen directly in front of the participants who use a keypad to select the number that is different. The task is presented in a series of interleaved four (spatially) congruent blocks and four (spatially) incongruent blocks lasting 9 minutes and 20 sec. Performance was titrated to 60% accuracy by adjusting the inter-trial intervals, thus controlling for individual differences in task performance (see Sheu et al., 2012) for a detailed description of the task). To further induce stress, a video camera recorded participant performance, the experimenter marked performance in direct view of the participant, negative feedback was provided by a loud aversive noise and a faux leader board was displayed prominently in front of participants.

**Sub-maximal Exercise Task**

A modified YMCA sub-maximal cycle test was implemented to predict the participant's $VO_{2max}$ (Golding, Myers, & Sinning, 1989). The test consists of four, 2-min stages cycling on an electronically braked cycle ergometer (Lode, Corival), where workload increases incrementally. Heart rate and $VO_2$ were assessed during the final 10 seconds of each

2-min stage and were plotted against bike resistance. $VO_{2max}$ was calculated as $VO_2$ at age-predicted maximal HR (220-age) and the workload required to elicit 70% $VO_{2max}$ was extrapolated.

**Procedures**

Participants completed three separate sessions (submaximal exercise test, acute psychological stress only, acute psychological stress following exercise), completed at least two days apart (Mean = 5.12, SD, 2.75 days). The time of testing was held constant within participants to minimise the effects of diurnal variations. Session one, was always the sub-maximal exercise session. Sessions two and three involving psychological stress testing were counterbalanced with participants randomly assigned to order presentation.

**Submaximal exercise session.** Following informed consent, height and weight measurements were obtained, a HR monitor (Polar T31) was attached, as well as a facial mask for respiratory data collection. Participants then completed the modified YMCA protocol. Following this, the MSIT task was introduced and participants completed a 20-second practice trial.

**Stress sessions (sessions 2 and 3).** Participants were seated on the bike ergometer with the necessary cardiovascular and respiratory equipment and instructed to remain still for five minutes (adaptation period), followed by a formal 10-min baseline period. Blood pressure was obtained in minutes 2, 4, 6, 8 and 10 of the baseline period, and ECG and metabolic data were analysed during these minutes. Each stress session progressed as follows:

**Acute psychological stress only session.** Participants completed the MSIT whilst remaining seated on the bike without peddling. Blood pressure measurements were obtained on commencement of each block of trials and ECG and metabolic data were analysed during these periods.

**Acute psychological stress following exercise.** First, participants completed 10-min cycling at 70% of their $VO_{2max}$. An experimenter ensured participants were cycling at 70rpm by providing feedback if participants exceeded ± 5rpm. Participants then completed a 20-min seated rest period off the bike, followed by a 10-min baseline period, and the MSIT in an identical protocol to the stress only session on the bike.

Prior to completing the exercise and psychological stress tasks, standardised audio instructions were provided to participants about the upcoming task and a post-task questionnaire pack was completed upon cessation of the MSIT.

**Data Reduction and Statistical Analysis**

Cardiovascular measurements obtained during the baseline period immediately preceding each stress task were averaged to calculate separate pre-task baseline values (Trotman et al., 2018; Veldhuijzen van Zanten et al., 2002). A 5 time (min 2, 4, 6, 8 and 10) repeated measures MANOVA during respective baselines revealed no significant time effect for BP and HR measurements, thus supporting the use of average baseline values (Veldhuijzen van Zanten et al., 2002). Cardiovascular and respiratory measurements assessed during each block of the MSIT were averaged to create MSIT stress task values.

To examine possible sex differences, sex was included as a between subject factor. For all analyses, there were no main effects for gender apart from a MANOVA containing SBP, DBP, and HR. Although a significant multivariate effect for gender, Pillai's trace = .470, $F(2,34) = 15.06$, $p < .001$, $\eta p2 = .470$, there was no time × gender interaction or session × gender interaction, suggesting although women had significantly lower SBP and DBP, and higher HR than men, SBP, DBP and HR responses to the MSIT did not differ. Subsequently, male and female data was collapsed for all subsequent analyses.

To examine whether participants were exercising at 70% $VO_{2max}$, a 2 time (estimated 70% $VO_{2max}$, $VO_2$ during final 9 minutes of solo exercise) repeated measures ANOVA was performed. The final nine minutes (excluding minute one) was selected as participants began from resting $VO_2$, and one-minute was deemed sufficient to achieve the correct intensity. One individual was exercising at an incorrect $VO_2$ calculation and was therefore removed from all future data analysis, resulting in a final sample of 39 (20 males, 19 females). A 2 session (stress only, stress following exercise) ANOVA examined whether individuals were engaged in the MSIT during both sessions.

To explore whether acute exercise attenuated physiological responses to psychological stress, a 2 time (baseline, stress) × 2 session (stress only, stress following exercise) repeated measures MANOVA was conducted. Differences in the psychological data were examined with several 2 session (stress only, stress following exercise) repeated measures MANOVAs, due to violation of assumptions when all variables were analysed in the same model (Verma, 2015). As such, five separate MANOVAs were run for psychological data: 1) primary appraisals, 2) secondary appraisals 3) mood state, 4) anxiety intensity, 5) anxiety interpretation. All MANOVAs met the assumptions for running repeated measures MANOVAs (Verma, 2015). Significant multivariate effects were further inspected with univariate ANOVAs for each dependent variable. For all univariate ANOVAs, Bonferroni post-hoc analyses were run where appropriate and partial eta-square ($\eta_p^2$) was reported as a measure of effect size. Individuals with occasional technical errors (i.e., loss of ECG signal, n = 9) were removed from the respective analysis which is reflected with altering degrees of freedom. The alpha level was set at $p < .05$ for all analyses.

## Results

### Manipulation Check of Exercise Intensity and Stress Task Engagement

As reported in Table 3.1, average predicted $VO_{2max}$ was 41.4 ± 9.91 ml · $min^{-1}$. There was no significant difference between estimated 70% $VO_{2max}$ (28.88 ± 7.11 ml · $min^{-1}$) and the $VO_2$ recorded during the final 9 minutes of exercise (28.63 ± 6.72 ml · $min^{-1}$), $F(1,36) = .341$, $p = .56$, $\eta_p^2 = .009$. Mean $VO_2$ during the final 9 minutes of the solo exercise was 69.83 ± 4.77% of $VO_{2max}$ values, confirming that participants were exercising at an intensity representing 70% $VO_{2max}$. Participants were similarly highly engaged in the stress task in both the stress only session (M = 5.18 ± 0.95) and the stress following exercise session (M = 5.08 ± 0.97), $F(1,37) = 0.56$, $p = .46$, $\eta_p^2 = .015$.

**Effect of Exercise on Physiological Responses to Psychological Stress**

The SBP, DBP and HR MANOVA revealed a significant multivariate time effect, Pillai's trace = .566, $F(3,27) = 11.73$, $p < .001$, $\eta_p^2 = .566$, session effect, Pillai's trace = .411, $F(3,27) = 6.29$, $p = .002$, $\eta_p^2 = .411$, and session × time interaction, Pillai's trace = .554, $F(3,27) = 11.16$, $p < .001$, $\eta_p^2 = .554$. Inspection of univariate results revealed significant time effects for SBP, $F(1,29) = 36.46$, $p < .001$, $\eta_p^2 = .557$, DBP, $F(1,29) = 19.96$, $p < .001$, $\eta_p^2 = .408$, and HR, $F(1,29) = 13.60$, $p = .001$, $\eta_p^2 = .319$. Session effects were found for HR, $F(1,29) = 10.68$, $p = .003$, $\eta_p^2 = .269$, but not SBP, $F(1,29) = 0.91$, $p = .35$, $\eta_p^2 = .031$, or DBP, $F(1,29) = 0.15$, $p = .70$, $\eta_p^2 = .005$. Session × time interaction effects were observed for SBP, $F(1,29) = 11.93$, $p = .002$, $\eta_p^2 = .291$, DBP, $F(1,29) = 6.14$, $p = .019$, $\eta_p^2 = .175$, and HR, $F(1,29) = 35.05$, $p < .001$, $\eta_p^2 = .547$ (see Figure 3.1). Post-hoc analyses indicated that SBP during the stress following exercise session tended to be lower ($p = .056$) than the stress only session, and although DBP was lower, no significant effect was observed during stress. Systolic blood pressure and DBP increased from baseline to stress during both sessions and baseline values did not differ across sessions. In contrast, HR during the stress following exercise session was significantly higher at pre-stress baseline compared to the stress only

baseline, with no HR differences during the MSIT between sessions. Heart rate significantly increased from baseline to task during the stress only session only.

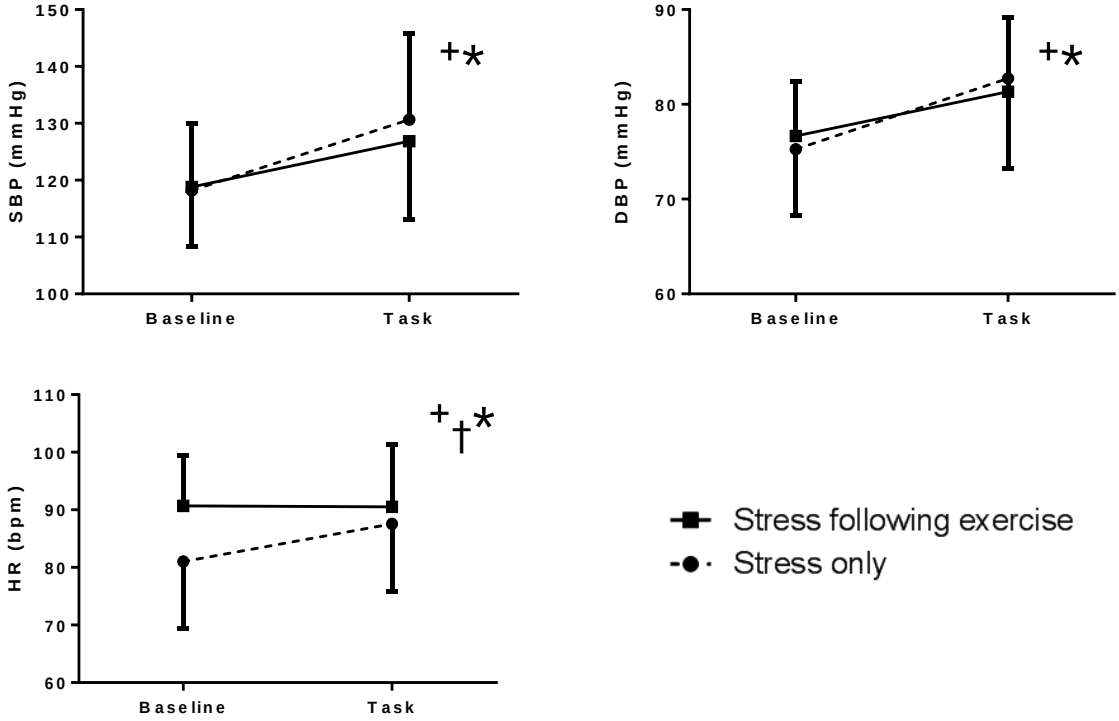


**Figure 3.1.** Mean (SD) systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) during baseline and stress task for stress only session, and stress following exercise session. +Significant time effect, $p$ <.05. †Significant session effect, $p$ <.05. *Significant interaction, $p$ <.05.

## Effect of Exercise on Psychological Responses to Psychological Stress

**Primary stress appraisal.** Primary stress appraisals revealed a multivariate session effect, Pillai's trace = .313, $F(4,35) = 3.98$, $p$ =.009, $\eta_p^2$ = .313, and univariate session effects were observed for perceived threat, $F(1,38) = 7.29$, $p$ =.010, $\eta_p^2$ = .161, perceived stressfulness, $F(1,38) = 13.25$, $p$ =.001, $\eta_p^2$ = .259, and perceived difficulty, $F(1,38) = 5.50$, $p$ =.024, $\eta_p^2$ = .126. No effect was evident for perceived demand, $F(1,38) = 2.15$, $p$ =.15, $\eta_p^2$

= .053 (see Table 3.2). Perceptions of threat, stressfulness, and difficulty were greater during the stress following exercise session compared to the stress only session.

**Secondary stress appraisal.** The secondary stress appraisal MANOVA revealed a multivariate session effect, Pillai's trace = .245, $F(2,37) = 6.02$, $p = .005$, $\eta_p^2 = .245$, however, no univariate session effects were found for perceived control or perceived ability to cope.

**Mood.** There was a multivariate session effect, Pillai's trace = .420, $F(6,33) = 3.99$, $p = .004$, $\eta_p^2 = .420$. Univariate analyses revealed that during the stress following exercise session, tension, $F(1,38) = 6.99$, $p = .012$, $\eta_p^2 = .155$, depression, $F(1,38) = 5.37$, $p = .026$, $\eta_p^2 = .124$, fatigue, $F(1,38) = 10.02$, $p = .003$, $\eta_p^2 = .209$, and confusion, $F(1,38) = 4.89$, $p = .033$, $\eta_p^2 = .114$, were greater, and vigour, $F(1,38) = 5.71$, $p = .022$, $\eta_p^2 = .131$, was lower (see Table 3.2). No univariate effect was found for anger, $F(1,38) = 0.16$, $p = .68$, $\eta_p^2 = .004$.

**Anxiety.** A multivariate session effect, Pillai's trace = .180, $F(2,37) = 4.06$, $p = .025$, $\eta_p^2 = .180$ was observed and univariate results revealed a significant session effect for somatic anxiety intensity, $F(1,38) = 6.54$, $p = .015$, $\eta_p^2 = .147$ (see Table 3.2). Somatic anxiety was lower during the stress following exercise session compared to the stress only session, with no effects evident for cognitive anxiety $F(1,38) = 0.21$, $p = .65$, $\eta_p^2 = .006$. No significant MANOVA effects were found for anxiety interpretation, Pillai's trace = .002, $F(2,36) = 0.04$, $p = .96$, $\eta_p^2 = .002$.

## Discussion

The results demonstrate that following a short bout (10-min) of high intensity exercise, systolic blood pressure responses to stress were attenuated, replicating results reported in a meta-analysis (Hamer et al., 2006). Exercise also reduced HR reactivity; however this appears to be a result of an altered baseline value, which is commonly observed due to delayed recovery following exercise (Michael, Graham, & Favis, 2017). As such, the

**Table 3.2.** Mean (SD) psychological data for the stress only and stress following exercise.

| | Post-task | | | |
|---|---|---|---|---|
| | Stress only | Stress following exercise | Multivariate *p*-value | Univariate *p*-value |
| **Mood State** | Mean ±SD | Mean ±SD | .004** | |
| Tension | 2.87 (2.08) | 3.90 (2.78) | | .012* |
| Depression | 0.59 (0.91) | 0.92 (1.44) | | .026* |
| Anger | 2.15 (2.87) | 2.31 (2.84) | | .683 |
| Fatigue | 0.87 (1.38) | 2.05 (2.63) | | .003** |
| Confusion | 4.15 (1.65) | 4.67 (2.24) | | .033* |
| Vigour | 4.51 (3.95) | 3.26 (3.05) | | .022* |
| **Anxiety Intensity** | | | .025* | |
| Cognitive Anxiety Intensity | 3.51 (1.28) | 3.62 (1.57) | | .648 |
| Somatic Anxiety Intensity | 2.69 (1.16) | 2.21 (1.23) | | .015* |
| **Anxiety Interpretation** | | | .961 | |
| Cognitive Anxiety Interpretation | –0.74 (1.50) | –0.76 (1.44) | | .917 |
| Somatic Anxiety Interpretation | –0.32 (1.29) | –0.26 (1.30) | | .824 |

| | Stress only | Stress following exercise | Multivariate *p*-value | Univariate *p*-value |
|---|---|---|---|---|
| **Primary Stress Appraisals** | | | .009* | |
| Perceived Threat | 0.81 (1.21) | 1.23 (1.47) | | .010** |
| Perceived Task Demands | 3.97 (1.09) | 4.31 (1.00) | | .151 |
| Stressful | 3.26 (1.25) | 3.82 (1.21) | | .024* |
| Difficulty | 3.79 (1.03) | 4.21 (1.08) | | .001** |
| **Secondary Stress Appraisals** | | | .005** | |
| Perceived Coping Resources | 3.54 (1.10) | 3.23 (0.99) | | .129 |
| Perceived Control | 3.64 (1.46) | 4.00 (1.47) | | .156 |

* $p$ <.05. ** $p$ <.01. ***$p$ <.001.

current results support our hypothesis and the adaptive effects of acute exercise prior to psychological stress in attenuating physiological stress responses.

This was the first study to investigate the effects of exercise on the cognitive appraisal of stress. Following exercise, primary appraisals (i.e., perceived threat, stressfulness and difficulty) were increased. Given that performance of the task was titrated within and between individuals, these alterations in perceived threat, difficulty and stress of the task are likely to be due to the exercise. Interestingly, perceived task demand (primary appraisal) did not differ across sessions, which may explain why the secondary appraisals were not altered. That is, although the task was more threatening, stressful and difficult, individuals did not perceive it as more demanding, and therefore perceived similar coping abilities and control. Future research should explore the impact of different exercise intensities on primary and secondary appraisals. As this is the first study to examine the impact on cognitive appraisals, it is unknown whether the primary appraisals were increased as a result of completing two consecutive demanding tasks or whether they increased as a direct result of the high intensity exercise. Understanding the mechanisms between exercise and appraisals will allow guidelines to be developed to undertake the appropriate exercise to reduce primary appraisals and improve the ability to cope with stress. Given the fundamental role that appraisals play in generating the stress response (Gianaros & Jennings, 2018), if negative appraisals are reduced, this could have follow-on benefits for the physiological and psychological stress responses. However, this hypothesis warrants further study.

The results demonstrate that in general, high intensity exercise has a maladaptive effect on mood state during subsequent stress, with increased scores on negative mood subscales, and lower positive vigour reported. While previous research has demonstrated equivocal results, the present findings further support the notion that this is likely due to the

exercise intensity. Indeed, while stationary cycling has predominantly been used, the intensity of cycling ranged from low intensity (Benvenutti et al., 2017; Roy & Steptoe, 1991) to high intensity (Duda et al., 1988; McGowan et al., 1985; Steptoe et al., 1993). Following low intensity cycling, reductions in tension and confusion were observed (Roth, 1989), whereas following high intensity cycling, greater tension and depression were found (Steptoe et al., 1993), with null effects also observed during high (McGowan et al., 1985) and low intensity (Roy & Steptoe, 1991). A recent study demonstrated that those assigned to low intensity exercise (i.e., walking or jogging) anger and anxiety did not increase in response to acute stress. In contrast, in those allocated to a no exercise control group the same stress event induced increases in anger and anxiety (Edwards, Rhodes, & Loprinzi, 2017). It was therefore proposed that low intensity exercise may be effective in preventing negative emotional states (Edwards et al., 2017). This aligns with preliminary animal data showing that low intensity exercise is the optimal intensity in stimulating the serotonergic pathway which has been implicated in positive mood states (Basso & Suzuki, 2017). Consequently, while high intensity exercise appears to be beneficial for cardiovascular responses to stress, it seems to be detrimental to mood responses to stress.

It is possible the negative effects of the exercise are due to the proximity (i.e., 30mins) between stress and high intensity exercise. Indeed, during and immediately following high intensity exercise, increases in negative emotions and perceived stress have been reported compared to moderate intensity sessions (Saanijoki et al., 2015). The aim of the current study was to use an exercise and stress paradigm which is known to reduce stressor-evoked blood pressure reactivity, and extend the findings to understand how acute exercise impacts psychological state during stress. Given that studies examining blood pressure have implemented a 30-min window, which allows physiological activity to recover to baseline

levels (Alderman et al., 2007), it was appropriate to maintain this time period so the effects on psychological state could be explored. This timeframe could limit the generalisability of results into real-life situations, and research should examine whether a longer timeframe between exercise and stress has less detrimental and possibly even beneficial effects on psychological responses to stress.

Exercise did reduce somatic anxiety levels. This is contrary to our hypothesis and somewhat surprising given the previous work demonstrating an increase following high intensity exercise (Duda et al., 1988). It is noteworthy that the reductions in somatic anxiety symptoms were in line with reductions in blood pressure responses (albeit not significantly related). The possibility that individuals accurately perceived reductions in blood pressure reflected by reductions in somatic anxiety is interesting. However, given that HR was higher during baseline following exercise, accurately perceiving small BP reductions while not being aware of heightened HR is an unlikely mechanism. A more likely explanation is that individuals became desensitised to the associated physiological symptoms of anxiety following acute exercise. Exposure to somatic anxiety symptoms such as elevations in HR in the absence of fear (i.e., exposure due to exercise), lowers negative perceptions of somatic symptoms experienced during non-exercise periods of somatic activation i.e., stress (Craske, Treanor, Conway, Zbozinek, & Vervilet, 2014). Repeated as well as single session exposures of exercise (Broman-Fulks, Kelso, & Zawilinski, 2015) are effective in reducing anxiety in individuals with elevated anxiety sensitivity (not relating to psychological stress). Consequently, following high intensity exercise, a similar process may have occurred with participants in the present study.

It is important to note that although somatic anxiety was reduced, these effects did not transfer to cognitive anxiety or alter the perceived interpretation of anxiety symptoms. It

might be expected that with reduced somatic anxiety, individuals would perceive their symptoms as more facilitative, and also experience a corresponding reduction in cognitive anxiety (Williams, Carroll, Veldhuijzen van Zanten, & Ginty, 2016). However, the beneficial effects appear specific to somatic anxiety. It could be that the increase in primary appraisals and negative mood may offset any potential benefits for cognitive anxiety and anxiety symptom interpretations. The current study extends the literature by demonstrating that acute exercise may be an effective technique to reduce symptoms of somatic anxiety during subsequent periods of psychological stress.

The current study has some important implications. Findings demonstrate that for stress regulation, although blood pressure reactivity is attenuated, high intensity exercise, with the exception of somatic anxiety, is detrimental to the subsequent psychological appraisals and responses to stress. From an applied point of view, it would appear that individuals with an upcoming stressful situation such as a presentation should avoid any high intensity exercise in the 30-mins prior to engaging in this stressful situation. It is also important that future work examine the effects of longer time periods between the exercise and stress exposure as well as how different intensity exercise bouts impact the psychological responses to upcoming stressful situations. Additionally, future research should examine how these factors influence performance on tasks in addition to psychological appraisals. This information will support the development of strategies to help regulate the negative psychological responses to stress.

The current study is not without limitations. While a strength of the work is both men and women were included, this study used healthy university students. Whether results would be similar in individuals with psychological disorders and/or an older population would be of interest for future work. Second, while the exercise intensity was selected a priori based on

existing literature demonstrating high intensity exercise to have the greatest effect on blood pressure reductions, some may consider implementing only high intensity exercise to be a limitation. A growing body of research supports the notion that exercise performed at low intensity or self-selected workloads yields the greatest benefit for psychological health (Edwards et al., 2017; Ekkekakis, 2009). Third, a strength to the current study is the within-subject (counterbalanced) repeated measure design; however, it is possible that participants may have experienced cross-session fatigue effects. Consequently, future work should examine the detailed physiological and psychological responses to stress following a range of exercise intensities as well as at self-selected workloads, using both within- and between-subject designs.

In summary, the present study demonstrated that although engaging in an acute bout of high intensity exercise resulted in a reduction in systolic blood pressure responses and somatic anxiety symptoms to stress, exercise had detrimental effects on primary stress appraisals and mood. Exercise had no effect on secondary appraisals. These findings demonstrate the maladaptive consequences of engaging in an acute bout of high intensity exercise on psychological appraisals and responses to stress. Future work exploring different exercise intensities and different lengths of time between the exercise and stress is needed to fully understand the impact of exercise on psychological appraisals and responses to stress.

# CHAPTER 4

## ASSOCIATIONS BETWEEN HEART RATE, PERCEIVED HEART RATE, AND ANXIETY DURING ACUTE PSYCHOLOGICAL STRESS.

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## Chapter 4 Preface

Chapter 3 demonstrated that engaging in an acute bout of high intensity exercise prior to exposure to acute psychological stress has a beneficial effect by attenuating blood pressure reactivity and somatic anxiety. However, the exercise primarily has a negative effect on psychological responses, including primary and secondary appraisals and mood states. An interesting observation is that although acute exercise is a physiological intervention, alterations in both psychological and physiological stress systems were demonstrated, and this was generally in opposing directions. In addition, when exploring the multidimensional measures of anxiety (cognitive and somatic anxiety), findings were not consistent for both anxiety constructs. That is, reductions in somatic anxiety during stress following exercise were reported, yet no changes to cognitive anxiety were observed. The reductions in somatic anxiety may have occurred due to a desensitisation to the cardiovascular sensations experienced following the acute exercise. This raises important questions regarding the role of physiological and psychological factors, in particular the perception of physiological responses and their contribution towards the experience of cognitive and somatic anxiety. As a result, Chapter 4 examined how heart rate reactivity and perceived heart rate change during acute psychological stress contribute towards the experience of cognitive and somatic anxiety.

## Abstract

Acute psychological stress elicits increases in heart rate (HR) and anxiety. Theories propose associations between HR, perceived HR, and anxiety during stress. However, anxiety is often measured as a unidimensional construct which limits a comprehensive understanding of these relationships. This research explored whether HR reactivity or perceived HR change was more closely associated with cognitive and somatic anxiety during acute psychological stress. Two laboratory-based studies were conducted. In a single laboratory session, healthy male (N = 71; study 1) and female (N = 70; study 2) university students completed three laboratory psychological stress tasks (counterbalanced), each with a preceding baseline. Heart rate, perceived HR change, and cognitive and somatic anxiety intensity and interpretation of anxiety symptoms were assessed immediately following each task. Data were aggregated across tasks. Results revealed actual HR change was unrelated to anxiety intensity, but was associated with more debilitative interpretations of anxiety (study 2). Perceptions of HR change were consistently associated with greater intensity of cognitive (study 1) and somatic (study 1 and 2) anxiety. Taken together, perceived HR rather than actual HR is more closely associated with anxiety intensity during psychological stress. The findings have implications for stress management and the clinical treatment of anxiety symptoms.



***Keywords:*** Acute psychological stress, Heart rate, Perceived heart rate, Cognitive anxiety, Somatic anxiety, Anxiety interpretation.

## Introduction

Anxiety is a common psychological disorder with lifetime prevalence as high as 28.8% in the United States (Bandelow & Michaelis, 2015; Kessler et al., 2005). Psychological stress plays a substantial role in anxiety. Stressful life events are known to precede anxiety disorders (Faravelli & Pallanti, 1989) and individuals with anxiety disorders report feeling more psychological stress and are impacted more by events that are considered stressful (Grossman et al., 2001; Mauss, Wilhelm, & Gross, 2004). It has been proposed that appraising a situation as threatening or stressful, combined with the alterations in cardiovascular activity, such as increases in heart rate (HR), may consequently lead to greater feelings of anxiety intensity (Jones et al., 2009; Mallorqui-Bague, Bulbena, Pailhez, Garfinkel, & Critchley, 2016).

Whilst influential theories propose a relationship between the mind and body (Damasio, 1996; James, 1884; Schachter & Singer, 1962), extensive experimental research has demonstrated limited associations between physiological and psychological responses to stress (Campbell & Ehlert, 2012). Rather, it has been proposed that the perception of the physiological changes (i.e., perceptions of HR change) may contribute to anxiety symptoms (Mallorqui-Bague et al., 2016). Evidence from clinical populations supports this (Mauss et al., 2004; Schmitz, Blechert, Kramer, Asbrand, & Tuschen-Caffier, 2012). For example, Mauss et al. (2004) found that anxiety reported during a speech task was associated with perceptions of physiological responses (e.g., greater HR, sweaty palms), while being unrelated to all actual physiological responses (Mauss et al., 2004). Another study showed that individuals who were led to believe their HR had increased, reported greater anxiety and physical cues of anxiety measured with the autonomic perception questionnaire (Wild, Clark, Ehlers, & McManus, 2008). This result was consistent across both high and low anxiety groups,

showing that individuals who perceive greater HR activity likely experience increased anxiety levels (Wild et al., 2008). As such, it could be suggested that perceived rather than actual HR is likely to be more strongly associated with anxiety responses to stress, however this is yet to be examined in a non-clinical population. Understanding which factors strongly contribute to the experience of anxiety symptoms, particularly in a healthy population, will inform interventions to minimise anxiety experienced during stress.

A limitation of the previous work examining the relationship between actual and perceived HR and anxiety is the anxiety assessment. When examining anxiety comprehensively, it is important to consider its multidimensional components, including cognitive and somatic anxiety (Degood & Tait, 1987; Martens, Vealey, & Burton, 1990). Cognitive anxiety refers to the mental symptoms of anxiety such as negative thoughts, whereas somatic anxiety is specific to the physical symptoms of anxiety such as increased HR (Martens, Vealey, et al., 1990). Previous work has typically assessed anxiety as a unidimensional construct (Anderson & Hope, 2009; Mauss et al., 2004; Wild et al., 2008) and often used scales specifically designed for the study (Grossman et al., 2001). Consequently, the lack of a relationship between HR and anxiety responses described in previous studies could be due to the lack of detailed and comprehensive anxiety measures used which separately tease out the distinction between cognitive anxiety and somatic anxiety.

When exploring the associations between anxiety and physiological responses to stress, it is logical to propose that compared to cognitive anxiety, somatic anxiety is more closely associated with HR responses given that its definition encompasses increases in HR (Martens, Vealey, et al., 1990). Central to anxiety disorders is the concept that individuals experience heightened physiological symptoms (i.e., palpitations) and focus their attention on bodily responses in stressful situations (American Psychiatric Association, 1994; Siess,

Blechert, & Schmitz, 2014). It would be of interest to explore the variance of cognitive and somatic anxiety during stress accounted for by actual physiological responses (HR) and perceived changes in HR in healthy individuals. Given the fundamental physiological components of somatic anxiety, it may be that HR and/or perceived HR responses will be associated with somatic anxiety during stress. In contrast, cognitive anxiety may be unrelated to perceptions of HR, and this discrepancy between anxiety constructs could underlie the equivocal findings in previous work. As such, understanding the relationship between HR, perceptions of HR, and cognitive and somatic anxiety in healthy individuals may highlight mechanisms contributing to the development of anxiety which can be used to develop appropriate preventative treatments.

In addition to its intensity, anxiety can also vary in whether it is interpreted as being facilitative or debilitative (Chamberlain & Hale, 2007; Jones, 1995; Jones et al., 1994; Thomas et al., 2002). Theories support the notion that anxiety is not always negative, and increases in anxiety do not always mean the anxiety will be more debilitative (Hanin, 2000; Neil, Wilson, Mellalieu, Hanton, & Taylor, 2012; Yerkes & Dodson, 1908). For example, the inverted-U hypothesis suggests a non-linear relationship between anxiety and perceived interpretation of these symptoms, in particular for somatic anxiety whereby elevated levels to an extent are thought to be beneficial (Martens, Burton, et al., 1990; Yerkes & Dodson, 1908). Similarly, updated theories such as the individual zones of optimal functioning propose anxiety is not always detrimental and that there are inter-individual differences in how anxiety symptoms are interpreted (Hanin, 2000). As such, it would be of interest to explore whether actual HR or perceptions of HR change relate to the both anxiety intensity but also the perceived interpretation of anxiety symptoms during psychological stress. Given that individuals with greater anxiety sensitivity report greater somatic and panic symptoms (Eley,

Stirling, Ehlers, Gregory, & Clark, 2004) it might be expected that individuals who perceive greater somatic symptoms (reflected in greater perceived HR changes during stress), report more debilitative anxiety symptoms, however this is yet to be examined. Understanding the factors contributing to the interpretation of anxiety symptoms will help develop targeted interventions which will reduce an individual's focus on negative cues, resulting in anxiety being interpreted as less debilitative and stress having a less maladaptive effect.

Research investigating psychological and physiological responses during stress typically use a single task, focussing on the social evaluative speech task (Cohen et al., 2000; Fox, Cahill, & Zougkou, 2010; Jansen, Gispen-de Wied, & Kahn, 2000; Mauss et al., 2004). An individual's cardiovascular and psychological responses vary depending on the type of stress (Kamarck & Lovallo, 2003). Even within the lab, cardiovascular and anxiety responses vary based on the nature and demands of the stress task (Richter, Friedrich, & Gendolla, 2008; Veldhuijzen Van Zanten et al., 2004; Wright, Killebrew, & Pimpalapure, 2002). For example, reaction time tasks and mental arithmetic tasks typically induce β-adrenergic driven cardiovascular responses, mirror tracing and cold pressor tasks typically produce α-adrenergic driven cardiovascular responses, and video games a mixed pattern of α-adrenergic and β-adrenergic cardiovascular responses (Allen, 2008; Kamarck & Lovallo, 2003; Turner, 1994). Differences in cognitive and somatic anxiety and stress appraisals are also evident between speech, mental arithmetic and competitive stress tasks (AlAbsi et al., 1997; Trotman et al., 2018). Consequently, previous relationships or lack of relationships between anxiety and actual and perceived HR changes may have been dictated by the type of stress task utilised in the study.

To provide a more valid and reliable measure of cardiovascular reactivity (including HR change), Kamarck et al. (1992) exposed individuals to several psychological stress tasks

with different demands, and aggregated data resulting in a 'trait' cardiovascular reactivity score. Literature has since called for studies to implement a variety of individual tasks and aggregate data to reduce idiosyncratic measurement variability resulting in a more reliable 'trait' overall score, thus increasing the generalisability of studies to general life stress (Kamarck, Jennings, Stewart, & Eddy, 1993; Kamarck & Lovallo, 2003). We are unaware of any literature calculating aggregated stressor-evoked anxiety and perceptions of HR change scores. However, it is likely that calculating aggregated psychological responses to several stress tasks will also provide a more reliable reflection of an individual's psychological response to acute psychological stress.

Literature has demonstrated gender differences in anxiety disorders, with increased severity and prevalence reported in females (McLean, Asnaani, Litz, & Hofmann, 2011). Furthermore, anxiety sensitivity (i.e., the fear of anxiety related sensations) is also much more prevalent in females compared with males (Armstrong & Khawaja, 2002; Norr, Albanese, Allan, & Schmidt, 2015; Stewart, Taylor, & Baker, 1997). Even though there have been mixed findings regarding gender differences in reactivity, a meta-analysis revealed stressor-evoked HR reactivity does not differ across genders (Brindle et al., 2014). However, literature has demonstrated females report greater anxiety, somatic complaints and cardiac associated complaints during psychological stress (Grossman et al., 2001). Given trait and state gender differences, it is likely that gender also influences the associations between our independent and dependent variables. Consequently, the present body of research first explored in a male sample whether relationships were apparent between perceived HR, actual HR and multidimensional anxiety outcomes. We then looked to replicate study 1 with a secondary study (study 2) to explore if the observed relationships could be extended and reproduced in an independent female sample.

The purpose of this two-study paper is to rigorously explore the associations between actual HR reactivity and perceived HR changes with cognitive and somatic anxiety intensity and symptoms interpretation in healthy male and female individuals. Three different stress tasks with a range of psychological demands and diverse stress responses were used. Aggregated HR reactivity, perceived HR change, cognitive and somatic anxiety intensity and symptom interpretation scores score were derived.

## Study 1

### Aims and Hypotheses

The aim of study 1 was to examine whether objectively measured HR reactivity or perceived HR changes to three aggregated stress tasks are predictors of 1) cognitive anxiety intensity, 2) somatic anxiety intensity, 3) cognitive anxiety symptom interpretation, 4) somatic anxiety symptom interpretation, experienced during acute psychological stress. It was hypothesised that perceptions of HR change would be more strongly associated with anxiety intensity than actual HR reactivity. Second, it was hypothesised perceptions of HR would be associated with more debilitative anxiety.

### Method

#### Participants

Participants were 71 healthy male university students (M [SD] age = 20.13 [1.12] years, M [SD] BMI = 22.78 [2.57] $kg/m^2$) who were non-smokers, had no history of cardiovascular disease, and had not taken any prescribed medication in the 4 weeks prior to testing. Participants were asked to abstain from consuming caffeine and eating up to 2 hours before testing as well as consuming alcohol and participating in vigorous exercise 12 hours before testing. All participants provided written informed consent and were given undergraduate module credit upon study completion. The study was approved by the

University of Birmingham ethics committee and data was collected September 2015 – March 2016.

**Cardiovascular Measures**

Heart rate was recorded continuously using the ambulatory monitoring system, VU-AMS5fs (TD-FPP, Vrije Universiteit, Amsterdam, the Netherlands; de Geus et al., 1995; Willemsen, DeGeus, Klaver, VanDoornen, & Carroll, 1996) to measure the electrocardiogram. Following automated inter-beat interval time series detection, ectopic beats were removed and the ECG was manually inspected.

**Questionnaires**

**Cognitive and somatic anxiety intensity and interpretation.** The Immediate Anxiety Measures Scale (IAMS; Thomas et al., 2002) assessed cognitive and somatic anxiety intensity as well as perceived interpretation of cognitive and somatic anxiety symptoms. Single items assessed cognitive and somatic anxiety intensity from 1 (not at all) to 7 (extremely), as well as the perceived interpretation from -3 (very debilitative/negative) to +3 (very facilitative/positive). The IAMS provides valid and reliable cognitive and somatic anxiety scores which have been validated against multi-item anxiety questionnaire (CSAI-2) (Thomas et al., 2002). In particular, validation has emphasised the measurement accuracy in close proximity to a stressful event, and the IAMS is used frequently in stress research to assess task specific anxiety (Moore, Vine, Wilson, & Freeman, 2012; Trotman et al., 2018; Williams et al., 2017).

**Perceived heart rate change.** Perceived HR change was assessed with a single item developed for the purpose of this study. Participants were asked to rate how much they perceived their HR to change during the acute psychological stress tasks from 0 (not at all) to 6 (very much so). A 7-point Likert-type scale was developed in line with commonly reported

stress-related psychological variables in the psychophysiology literature (Bibbey, Carroll, Roseboom, Phillips, & de Rooij, 2013; Ginty, Carroll, & Williams, 2014). Given validation literature supporting the use of single item measures to assess psychological states in close proximity to a stressful event (Thomas et al., 2002) and that recovery of HR occurs transiently following stress, a visual analogue scale was implemented to limit any temporal effects which could bias the reporting.

**Manipulation checks.** Following completion of each task, participants rated how difficult and stressful they found the task, as well as the extent they were trying to perform well as a measure of engagement. Each question was assessed using a 7-point Likert scale from 1 (not at all) to 7 (extremely).

**Acute Psychological Stress Tasks**

Three standardised acute psychological stress tasks were implemented which have been shown to elicit a diverse set of cardiovascular and anxiety responses (AlAbsi et al., 1997; Allen, 2008; Turner, 1994). The chosen tasks were selected to represent various stressors that individuals encounter on a daily basis which vary in the demands required to be able to cope to enhance lab-to-life generalisability. The selected validated stressors include social evaluative negative feedback (e.g., work presentations), competitive pressure (e.g., competition for job applications), and perseverance (e.g., problem solving). Task responses were aggregated across these three domains to generate a 'trait' response per individual, increasing reliability and generalisability of results (Kamarck et al., 1992; Kamarck et al., 1993; Kamarck & Lovallo, 2003). The following psychological tasks were used:

**Mental arithmetic stress.** Participants completed a 6-minute version of the *Paced Auditory Serial Addition task* (PASAT; Gronwall, 1977) whereby a series of single-digit numbers were presented through audio speakers. Participants add consecutive numbers

together and verbalise their answers, whilst remembering the most recent number to add it to the next presented number. The presentation frequency of the numbers decreased from 2.0s to 1.6s and 1.2s every 2 minutes. The PASAT involves social evaluation; participants were videotaped, which was displayed on a monitor, a mirror was presented directly in front of the participant in which they were instructed to watch themselves, and they were informed that 'body language experts' would assess their anxiety levels. Furthermore, a prominent leader board was displayed. Finally, participants were informed they would hear a loud buzzer if they hesitated or answered incorrectly, which has been shown to increase the stress experienced (Veldhuijzen Van Zanten et al., 2004). In reality, no such body language analysis took place and participants were buzzed at standardised intervals. The PASAT reliably perturbs the cardiovascular system (Ring et al., 2002; Veldhuijzen Van Zanten et al., 2004) and demonstrates strong test-retest reliability (Ginty et al., 2013).

**Competition task.** A computer car racing game (Need for speed: Underground – EA Games), was used as a competitive stress task. Participants controlled the game with their ring, middle and index fingers of their dominant hand, thus minimising physical exertion. The task involved completing 8-laps of a predetermined race track against 5 computerised opponents. The game manipulations allowed races to be standardised and the computerised opponents' ability to match that of the participants. A prominent leader board with the 5 quickest times in the study was displayed in front of the participants and they were informed that £10 in Amazon vouchers would be provided to the top performance at the completion of the study to emphasise the competitive nature of the task. On average, participants completed the task in 05:32min. Competition has frequently been utilised in stress psychophysiology research (Turner, 1994), has been recommended for inclusion in a behavioural stress battery

(Turner et al., 1997), has previously demonstrated it elicits a cardiovascular stress response and elevates cognitive and somatic anxiety (Trotman et al., 2018).

**Puzzle task.** Participants were provided with six-minutes to complete a series of Euler puzzle tracing tasks. Specifically, participants had to trace directly over all the lines of a set puzzle, without removing their pen from the puzzle or tracing over a previously marked line. There were three puzzles to complete during the task, starting with a relatively easy puzzle (average time to complete = 0:28min), moving to a progressively harder puzzle (2:44min) and finishing with an impossible puzzle. An experimenter stood next to the participants to ensure that they completed the task correctly and were instructed to complete as many of the puzzles as possible within the 6-minutes. Unsolvable puzzle tasks have the fundamental principles of stress, including novelty, unpredictability and negative feedback are frequently used in stress literature (Perry, Calkins, Nelson, Leerkes, & Marcovitch, 2012; Yang et al., 2019). For a full description including diagrams of puzzles used, see Bibbey (2015).

**Procedures**

Following informed consent, height and weight measurements were obtained and ECG equipment was attached. Participants began a 10-min baseline period watching a nature documentary before completing the stress tasks which were presented in a counterbalanced order. Each task was preceded with its own respective 10-min baseline period and was set-up identically: 1) following basic task instructions participants completed a short practice to become familiar with the task demands, 2) standardised audio instructions were presented to emphasise the relative characteristics and manipulations of each task, and 3) participants completed the task. Immediately following each task participants provided their perceived HR change and completed the IAMS and manipulation checks. Participants were then detached from the physiological recording equipment, debriefed and thanked for their participation.

**Data Reduction and Statistical Analysis**

HR measurements taken during each rest period were averaged to yield a pre-task baseline value and minute-by-minute HR measurements taken during each task were averaged to calculate separate competition, mental arithmetic and puzzle task values. Subsequently, reactivity for HR (stress task minus baseline) was computed. A 3 task (competition, PASAT, puzzle) by 2 time (baseline, task) repeated measures ANOVA was performed to confirm that each stress task perturbed increases in HR activity. Psychological data was analysed using MANOVAs unless assumptions were violated. As such, a series of 3 task (competition, PASAT, puzzle) repeated measures ANOVAs were run to explore whether the 3 tasks differed regarding manipulation checks and perceived HR change. Two separate MANOVAs were run for anxiety intensity and for anxiety symptom interpretation, meeting MANOVA assumptions (Verma, 2015). Heart rate reactivity scores were standardised for each task, and standardised scores aggregated across the three tasks to create an aggregate HR reactivity score (Kamarck et al., 1992; Kamarck & Lovallo, 2003). Similarly, for perceived HR, cognitive and somatic anxiety intensity and perceived interpretation, scores were standardised and averaged across tasks to create an aggregate score for each separate variable. A Pearson's correlation explored whether actual HR reactivity associated with perceived HR change. Heart rate reactivity scores and anxiety scores were analysed as these represent the values during the actual stress period. To determine associations between HR reactivity, perceived HR change and anxiety scores, separate multiple regressions were run for each anxiety construct. Aggregate HR reactivity and aggregate perceived change in HR were entered simultaneously to predict each anxiety response. For ANOVAs conducted, Greenhouse-Geisser values were reported, Bonferroni post-hoc analyses were run where appropriate, and partial eta-square was implemented as a measure of effect size. All data was analysed in SPSS

(version 22) where data was screened and participants with outliers (n = 4) greater than 3 standard deviations were removed from all analyses (Trotman, Gianaros, Veldhuijzen Van Zanten, Williams, & Ginty, 2018). Therefore, a sample of 67 participants was analysed. No participants dropped out from the study, however missing data due to cardiovascular equipment malfunction ($n$ = 6) or failure to complete a questionnaire ($n$ = 2) is reflected by variations in degrees of freedom for the respective analyses. The alpha level was set at $p$ <.05 for all analyses.

## Results

### Task Comparison Manipulation Checks

**Task ratings.** A 3 task (PASAT, competition, puzzle) ANOVA revealed all three tasks were rated as moderately to highly difficult, stressful and engaging. Significant task effects were observed for perceived difficulty ($n$ = 67), $F(2,114) = 52.20$, $p$ <.001, $\eta^2$ =.445, and perceived stressfulness ($n$ = 67), $F(2,125) = 34.59$, $p$ <.001, $\eta^2$ =.347. The PASAT was rated as the most difficult, followed by the puzzle, and then the competition. Similarly, the PASAT was rated as the most stressful, however no differences between the competition and the puzzle were evident. No task effects were observed for task engagement ($n$ = 67), $F(2,138) = 0.98$, $p$ = .377, $\eta^2$ =.014 (see Table 4.1).

**Heart rate.** A 3 task (PASAT, competition, puzzle) by 2 time (baseline, stress) ANOVA ($n$ = 61) revealed a significant task effect, $F(2,112) = 26.48$, $p$ <.001, $\eta^2$ = .306, a significant time effect, $F(1,60) = 139.97$, $p$ <.001, $\eta^2$ = .700, and a significant task by time interaction, $F(2,109) = 48.13$, $p$ <.001, $\eta^2$ = .445 (see Table 4.1). Post-hoc analyses revealed that HR increased from baseline during each task. No task differences were evident during baseline, but HR was significantly greater during the competition and the PASAT compared to the puzzle task.

**Perceived heart rate change.** A 3 task (PASAT, competition, puzzle) ANOVA ($n$ = 66) revealed a significant task effect for perceived HR, F(2,127) = 45.04, $p$ <.001, $\eta^2$ =.409. The greatest perceived change in HR was during the PASAT, followed by the competition which was in turn greater than the puzzle task.

**Anxiety.** Two separate MANOVAs (anxiety intensity and anxiety interpretation; $n$ = 66) revealed a significant multivariate task effect, for cognitive and somatic anxiety intensity, Pillai's trace = .700, $F(4,62) = 36.08$, $p$ <.001, $\eta_p^2$ = .700, and cognitive and somatic anxiety interpretation, Pillai's trace = .377, $F(4,62) = 9.39$, $p$ <.001, $\eta_p^2$ = .377. Inspection of

**Table 4.1.** Study 1- Mean (SD) heart rate, perceived heart rate change, anxiety and post-task appraisal scores during the three stress tasks.

| | PASAT | Competition | Puzzle |
|---|---|---|---|
| Heart rate during baseline (bpm) | 67.10 (10.75) | 67.48 (11.37) | 67.34 (10.84) |
| Heart rate during task (bpm) | 79.01 (12.65)[#] | 80.09 (12.29)[#] | 71.56 (10.26) [ab#] |
| Perceived heart rate change (0 – 6) | 4.48 (1.12) | 3.58 (1.42)[a] | 2.59 (1.30) [ab] |
| Cognitive anxiety intensity (1 – 7) | 5.26 (1.51) | 3.42 (1.54)[a] | 3.81 (1.56)[a] |
| Somatic anxiety intensity (1 – 7) | 4.24 (1.71) | 3.34 (1.56)[a] | 2.79 (1.39)[ab] |
| Cognitive anxiety interpretation (-3 – +3) | -1.21 (1.44) | 0.21 (1.34)[a] | -0.22 (1.43)[a] |
| Somatic anxiety interpretation (-3 – +3) | -0.80 (1.25) | 0.02 (1.32)[a] | -0.28 (1.22)[a] |
| Perceived stressfulness (1 – 7) | 5.48 (1.39) | 3.90 (1.47)[a] | 3.98 (1.52 [a] |
| Task difficulty (1 – 7) | 5.97 (0.99) | 3.90 (1.40)[a] | 4.95 (1.11)[ab] |
| Engagement (1 – 7) | 6.35 (1.04) | 6.43 (1.18) | 6.26 (1.04) |

[a]Significantly different at $p$ <.05 from PASAT, [b]Significantly different at $p$ <.05 from competition. [#]Significantly different from baseline.

univariate results revealed significant task effects for cognitive intensity, $F(2,117) = 42.48$, $p < .001$, $\eta^2 = .395$, somatic intensity, $F(2,127) = 29.46$, $p < .001$, $\eta^2 = .312$, cognitive symptom interpretation, $F(2,125) = 21.63$, $p < .001$, $\eta^2 = .250$, and somatic symptom interpretation, $F(2,117) = 10.28$, $p < .001$, $\eta^2 = .137$. Post-hoc analyses revealed that cognitive and somatic anxiety intensity were significantly greater during the PASAT compared to the competition and puzzle tasks (see Table 4.1). Lower somatic anxiety was reported during the puzzle task compared to the competition task. More debilitative interpretations of cognitive and somatic anxiety symptoms were reported during the PASAT compared to the competition and puzzle tasks.

**Main Analyses**

The following analyses are computed with the three stress task aggregated scores, with an individual value for each psychological and physiological variable.

**Association between heart rate reactivity and perceived heart rate change.** A Pearson's correlation revealed that perceived HR change and objectively measured HR reactivity were associated with each other, $r(58) = .276$, $p = .033$.

**Regression analyses predicting anxiety.** Significant regression models for cognitive, $F(2,56) = 11.12$, $p < .001$, and somatic, $F(2,56) = 9.77$, $p < .001$, anxiety intensity were evident, with perceived HR change being an independent predictor of these outcome measures, and actual HR reactivity being unrelated (see Table 4.2). Greater perceived increases in HR change, predicted greater cognitive and somatic intensity. Cognitive, $F(2,56) = 2.03$, $p = .141$, and somatic, $F(2,56) = 1.39$, $p = .256$, anxiety interpretations were not significantly predicted by objective HR reactivity or perceived HR change.

**Table 4.2.** Study 1 - Multiple regressions with aggregate perceived heart rate change and aggregate heart rate reactivity predicting anxiety.

| Criterion | Predictors | $R^2$ | β | *p* |
|---|---|---|---|---|
| Cognitive anxiety intensity | | .284*** | | |
| | Perceived heart rate change | | .542*** | <.001 |
| | Heart rate reactivity | | -.037 | .752 |
| Somatic anxiety intensity | | .259*** | | |
| | Perceived heart rate change | | .503*** | <.001 |
| | Heart rate reactivity | | .018 | .879 |
| Cognitive anxiety interpretation | | .068 | | |
| | Perceived heart rate change | | -.185 | .173 |
| | Heart rate reactivity | | .239 | .080 |
| Somatic anxiety interpretation | | .048 | | |
| | Perceived heart rate change | | -.199 | .147 |
| | Heart rate reactivity | | .157 | .252 |

*** $p$ <.001,

**Summary of Study 1 Results**

Objectively measured HR reactivity and perceived change in HR reactivity were significantly associated. As hypothesised, subjective perceptions of HR change, and not HR reactivity, was associated with cognitive and somatic anxiety intensity during acute psychological stress. Individuals who perceived a greater change in their HR experienced greater levels of cognitive and somatic anxiety intensity. It therefore appears that both somatic and cognitive components of anxiety are related to the perception of cardiac sensations rather than actual HR reactivity. Neither HR reactivity nor perceptions of HR change were associated with anxiety interpretation.

A limitation of study 1 is that the subjective perceived changes in HR were assessed in terms of a general change in HR without inferring any direction in this change. A growing

body of literature supports *the blunted reactivity* hypothesis (Phillips et al., 2013) where some individuals may experience a lower HR reactivity during stress. Indeed, the current study's data demonstrate HR reactivity ranged from -6.9 bpm to +36 bpm. As such, it is unknown whether a participant's reported perceived HR change represented a perceived increase or perceived decrease in HR. Accordingly, study 2 addressed these limitations by refining the measure of perceived HR change by adding a directional component (i.e., the extent to which the heart is perceived to be increasing or decreasing during stress) to examine if the associations change based on this alteration. Second, study 1 investigated the research question in males only, and is therefore limited in its generalisability. Several lines of research report gender differences in anxiety responses and perceived physiological changes during psychological stress (Grossman et al., 2001; Quigley, Barrett, & Weinstein, 2002). Consequently, this study aimed to extend the findings to a female sample, to explore if results were similar across genders.

## Study 2

### Aims and Hypotheses

Study 2 aimed to extend the findings of study 1 by investigating whether HR reactivity and/or perceived change in HR predicted cognitive or somatic anxiety responses during acute psychological stress in a separate sample consisting of female participants. It was hypothesised that HR reactivity would not be associated with cognitive or somatic anxiety, whereas perceiving greater increases in HR change would be positively associated with somatic anxiety intensity during acute psychological stress. Although not hypothesised in study 1, perceived HR changes were associated with cognitive anxiety intensity. As such, study 2 re-examined this relationship. It was hypothesised that whilst HR reactivity would

not, perceptions of HR change would be associated with somatic anxiety symptom interpretation, but not with cognitive symptom interpretation.

## Method

### Participants

Seventy healthy female university students participated in study 2 (M [SD] age = 19.71[0.95] years, M [SD] BMI = 22.28 [3.10] kg/m$^2$), with data collected September 2016 – March 2017. All participant exclusion criteria were identical to the study 1, except all participants in this sample were taking the contraceptive pill.

### Cardiovascular Measures

The measures were identical to those of study 1.

### Questionnaires

All questionnaires were identical to study 1, except perceived HR which was altered as described below.

**Perceived heart rate change.** Perceived HR was assessed with a single item asking individuals to rate their perceived change in HR on a 7-point Likert scale. Ratings ranged from -3 (Large decrease in HR), 0 (No change in HR), to +3 (Large increase in HR). This item was refined to acknowledge both increases as well as decreases in perceived HR during the acute psychological stress tasks.

### Acute Stress Tasks

Participants completed identical versions of the PASAT, competition and puzzle tasks. The average time to complete the competition task in this sample was 05:53min. The average time to complete puzzle 1 was 0:27min and puzzle 2 was 1:57min.

### Procedures and Data Analysis

The procedures and data analysis were identical to study 1. No participants dropped out from the study however three individuals were removed from all analyses due to being identified as outliers. Therefore, a sample of 67 participants was analysed. Missing data due to cardiovascular equipment malfunction ($n$ = 9) is reflected by variations in degrees of freedom for the respective analyses.

## Results

### Task Comparison Manipulation Checks

**Task ratings.** All three tasks were rated as moderately to highly difficult, stressful and engaging (see Table 4.3). A 3 task (PASAT, competition, puzzle) ANOVA revealed significant task effects for difficulty ($n$ = 67), $F(2,110) = 37.720$, $p < .001$, $\eta^2 = .364$, and stressfulness ($n$ = 67), $F(2,107) = 41.72$, $p < .001$, $\eta^2 = .387$. The PASAT was rated the most difficult, followed by the puzzle and then the competition. The PASAT was rated as the most stressful, with no differences reported between the competition and the puzzle. No differences in engagement were reported ($n$ = 67), $F(2,113) = 0.79$, $p = .43$, $\eta^2 = .01$.

**Heart rate.** A 3 task (PASAT, competition, puzzle) by 2 time (baseline, stress) ANOVA ($n$ = 58) revealed a significant task, $F(2,112) = 11.04$, $p < .001$, $\eta^2 = .162$, time, $F(1,657) = 99.19$, $p < .001$, $\eta^2 = .162$, and a significant task by time interaction, $F(2,108) = 32.65$, $p < .001$, $\eta^2 = .364$. Post-hoc analyses revealed for all tasks HR increased from baseline to stress. No task differences were evident during baseline but HR was greater during the competition and the PASAT compared to the puzzle task (see Table 4.3).

**Perceived heart rate change.** A 3 task (PASAT, competition, puzzle) ANOVA ($n$ = 67) revealed significant task effects for perceived HR change, $F(2,120) = 21.87$, $p < .001$, $\eta^2 = .249$. The greatest perceived change in HR was during the PASAT, followed by the competition with the puzzle task significantly lower than both tasks (see Table 4.3).

**Table 4.3.** Study 2 - Mean (SD) heart rate activity, perceived heart rate change, anxiety and post-task appraisal scores during the three stress tasks.

| | PASAT | Competition | Puzzle |
|---|---|---|---|
| Heart rate during baseline (bpm) | 69.03 (10.84) | 69.61 (11.65) | 69.97 (12.32) |
| Heart rate during task (bpm) | 79.03 (13.33)[#] | 79.08 (13.47) [#] | 73.35 (11.16)[ab#] |
| Perceived heart rate change (-3 – +3) | 1.85 (0.68) | 1.57 (0.87)[a] | 1.04 (0.84)[ab] |
| Cognitive anxiety intensity (1 – 7) | 5.33 (1.18) | 3.60 (1.50)[a] | 3.78 (1.62)[a] |
| Somatic anxiety intensity (1 – 7) | 4.51 (1.62) | 3.70 (1.61)[a] | 2.85 (1.41)[ab] |
| Cognitive anxiety interpretation (-3 – +3) | -1.12 (1.50) | -0.21 (1.31)[a] | -0.19 (1.28)[a] |
| Somatic anxiety interpretation (-3 – +3) | -0.96 (1.41) | -0.04 (1.29)[a] | -0.13 (1.16)[a] |
| Perceived stressfulness (1 – 7) | 5.58 (0.97) | 4.01 (1.37)[a] | 4.18 (1.53)[a] |
| Task difficulty (1 – 7) | 6.04 (0.84) | 4.64 (1.26)[a] | 5.18 (0.95)[ab] |
| Engagement (1 – 7) | 5.74 (1.00) | 5.72 (1.10) | 5.93 (0.89) |

[a]Significantly different at $p < .05$ from PASAT, [b]Significantly different at $p < .05$ from competition. [#]Significantly different from baseline.

**Anxiety.** Two separate MANOVAs ($n = 67$) revealed a significant multivariate task effect, for cognitive and somatic anxiety intensity, Pillai's trace = .747, $F(4,63) = 46.56$, $p < .001$, $\eta_p^2 = .747$, and cognitive and somatic anxiety interpretation, Pillai's trace = .376, $F(4,63) = 9.50$, $p < .001$, $\eta_p^2 = .376$. Inspection of univariate results revealed significant task effects for cognitive intensity, $F(2,124) = 62.37$, $p < .001$, $\eta^2 = .486$, somatic intensity, $F(2,131) = 29.65$, $p < .001$, $\eta^2 = .310$, cognitive symptom interpretation, $F(2,130) = 19.97$, $p < .001$, $\eta^2 = .25$, and somatic symptom interpretation, $F(2,131) = 14.13$, $p < .001$, $\eta^2 = .176$, were observed. Cognitive and somatic anxiety intensity were greater during the PASAT compared to the competition and puzzle tasks, with greater somatic anxiety intensity reported during the

competition compared to the puzzle task. More debilitative perceptions of cognitive and somatic anxiety were reported during the PASAT compared to the competition and puzzle tasks (see Table 4.3).

**Table 4.4.** Study 2 - Multiple regressions with aggregate perceived heart rate change and aggregate heart rate reactivity predicting anxiety.

| Criterion | Predictors | $R^2$ | β | *p* |
|---|---|---|---|---|
| Cognitive anxiety intensity | | .032 | | |
| | Perceived heart rate change | | .168 | .214 |
| | Heart rate reactivity | | .047 | .729 |
| Somatic anxiety intensity | | .131* | | |
| | Perceived heart rate change | | .290* | .026 |
| | Heart rate reactivity | | .188 | .142 |
| Cognitive anxiety interpretation | | .090 | | |
| | Perceived heart rate change | | -.022 | .863 |
| | Heart rate reactivity | | -.296* | .026 |
| Somatic anxiety interpretation | | .118* | | |
| | Perceived heart rate change | | -.218 | .092 |
| | Heart rate reactivity | | -.242 | .063 |

* $p < .05$

## Main Analyses

The following analyses are computed with the three stress task aggregated scores, with an individual value for each psychological and physiological variable.

**Association between heart rate reactivity and perceived heart rate change.** A Pearson's correlation revealed perceived HR change and objectively measured HR reactivity were not associated with each other, $r(58) = .109$, $p = .417$.

**Regression analyses.** A significant regression model for somatic anxiety intensity, $F(2,55) = 4.16$, $p = .021$, revealed perceived HR change was an independent predictor of

somatic intensity, and actual HR reactivity was unrelated. Greater perceived increases in HR were predictive of greater somatic anxiety intensity. The model for cognitive anxiety intensity, $F(2,55) = 0.91$, $p = .408$ was non-significant. The model for cognitive anxiety interpretation, $F(2,55) = 2.70$, $p = .076$, was non-significant, however actual HR reactivity individually predicted more debilitative cognitive anxiety interpretations (see Table 4.4). A significant model for somatic anxiety interpretation, $F(2,55) = 3.67$, $p = .032$ was evident, but neither predictors were individually significant.

**Summary of Study 2 Results**

Unlike study 1, there was a dissociation between objectively measured HR reactivity and subjective perceived change in HR. As hypothesised, perceptions of HR change were associated with somatic anxiety intensity and objectively measured HR reactivity was unrelated to anxiety intensity. In contrast to study 1, cognitive anxiety intensity was unrelated to perceived HR change. While the overall model for cognitive anxiety interpretation was non-significant, HR reactivity was individually associated with cognitive anxiety symptom interpretation. In contrast, the overall somatic anxiety model was significant but neither predictor was individually significant (HR reactivity approaching significance, $p = .06$). Thus, greater perceived increases in HR appear to be related to greater levels of somatic anxiety intensity and while greater objectively measured HR reactivity may be related to more debilitative interpretations of cognitive and somatic anxiety symptoms.

## General Discussion

Despite theories proposing associations between anxiety and physiological responses such as HR during stress (Damasio, 1996; James, 1884; Schachter & Singer, 1962), consistent evidence supporting a relationship between anxiety and HR is lacking (Campbell & Ehlert, 2012; Feldman et al., 1999). The present studies were the first to explore the associations

between HR reactivity and comprehensive assessments of anxiety by making a distinction between cognitive and somatic anxiety and examining both anxiety intensity and interpretation. Furthermore, by exposing participants to several psychological stress tasks and creating a 'trait' stress response score, this increased the reliability and generalisability of the stress responses. Whilst utilising this methodology, perceived HR responses during stress appear to be more closely related to anxiety than actual HR reactivity. Notably, the associations were not consistent across the male (study 1) and female sample (study 2), and associations were stronger in the male sample, which raises interesting questions about whether factors contributing to anxiety differ across genders, which should be explored with future research.

Research has found patients with high anxiety compared to low anxiety report greater perceived physiological activation and anxiety, which is independent of objectively measured physiological responses (Edelmann & Baker, 2002; Grossman et al., 2001). However, the current work is the first to specifically model both the actual HR response as well as perceived HR response and compare which of the two independently predicts cognitive and somatic anxiety levels. The results across both studies for somatic anxiety support the literature showing that general anxiety is associated with perceptions of physiological responses (e.g., HR, skin conductance, respiration) during psychological stress (Mauss et al., 2004). However, previous studies have utilised general anxiety measures which do not assess the multidimensional nature of cognitive and somatic anxiety, or the perceived interpretation of these symptoms. This is an important nuance, as understanding whether perceptions of physiological activity during periods of stress contribute to cognitive and somatic anxiety will aid the ability to prevent and treat the development of anxiety disorders. Anxiety theories have suggested augmented perceptions of cardiac sensations during stress can lead to

heightened anxiety (Clark et al., 1997; Mallorqui-Bague et al., 2016). It could be that a vicious cycle occurs, where perceiving greater changes in HR increases anxiety levels, consequently leads to greater awareness of heartbeats, exacerbating the somatic anxiety further (Clark et al., 1997; Mallorqui-Bague et al., 2016). Thus, interventions aimed at altering how an individual perceives their HR responses during stress, may reduce the anxiety and emotional distress experienced, and interrupt the negative cascade leading to greater experienced anxiety. However, due to the results being cross-sectional causation cannot be concluded.

Contrary to our hypotheses, perceived HR change positively associated with cognitive anxiety in the male sample. Anxiety is a complex disorder with experiential, behavioural and physiological components (Mallorqui-Bague et al., 2016). The interaction of anxiety antecedents, and specifically the awareness of physiological symptoms, may contribute to exacerbating cognitive components of anxiety. Indeed, it is established from the anxiety sensitivity literature that perceiving arousal sensations (e.g., HR) as negative, can lead to anxiety symptoms more akin to cognitive anxiety such as fear and other negative thoughts (Taylor, Jang, Stewart, & Stein, 2008). Furthermore, evidence has shown that exposing individuals to these arousal sensations in the absence of fear (i.e., exercise) can reduce anxiety levels (McEntee & Halgin, 1999). Thus, perceptions of HR during stress may be related to anxiety manifesting as both somatic and cognitive symptoms. It is important to note that in the male sample only there are moderate to large effect sizes for perceived changes in HR predicting cognitive and somatic anxiety ($\beta$'s > .50). This supports our hypothesis, that the perceptions of HR are associated with multidimensional symptoms of anxiety intensity. While it is unknown if these participants are misattributing their physiological symptoms, these effects emphasise the importance for clinicians to reduce the focus on physiological signals

during stress, which could contribute to the exacerbation of anxiety states (Mallorqui-Bague et al., 2016).

It is not clear why the association between perceived HR changes and cognitive anxiety was evident in males only. It has previously been shown that females report greater anxiety sensitivity, in particular relating to physical concerns (Stewart et al., 1997), experience greater severity of anxiety symptoms (McLean et al., 2011), and differ in their self-reported physiological symptoms (Grossman et al., 2001). However, in the current study, males and females reported similar levels of anxiety across studies (see Tables 4.1 and 4.3), with analyses confirming no gender differences (see supplementary Table 4.1). It is plausible that differences across studies were found due to the altered assessment in perceived change in HR, and thus interpretation of the current results must be made with caution across samples. Nonetheless, results suggest that in males, greater perceived HR change contributes not only to greater somatic anxiety, but also to greater cognitive anxiety. Future research should aim to develop a standardised methodology to assess perceptions of HR during stress.

This is the first study to implement measures of anxiety symptom interpretation and examine whether HR and/ or perceived HR relate to how anxiety is interpreted. Whilst no relationships were evident in the male sample, in the female sample actual HR reactivity was related to more debilitative cognitive symptoms, albeit with a small association, and there was a trend for an association with somatic anxiety symptoms. Interestingly, when analysing individual bivariate correlations (not multiple regressions), results demonstrated HR reactivity to be significantly associated with both cognitive ($r(56) = -.30$, $p = .02$) and somatic ($r(56) = -.27$, $p = .04$) anxiety interpretation. These data suggest that bodily signals contribute to the extent to which anxiety symptoms are interpreted as being facilitative or debilitative. That is, with greater HR reactivity during acute psychological stress, cognitive and somatic anxiety

symptoms are interpreted as more debilitative. These anxiety interpretation results align with anxiety models that emphasise physiological symptoms as important in anxiety states (Clark et al., 1997; Mallorqui-Bague et al., 2016). This could explain why few studies have found associations between anxiety and physiological responses; relationships with physiological activity may be specific to the anxiety interpretation rather than the intensity.

No such associations were evident in the male sample for anxiety interpretation. This could be due to reported gender differences in anxiety sensitivity (the fear of anxiety sensations) (Stewart et al., 1997). Indeed, females score higher on global anxiety sensitivity scales, specifically factors relating to physical concerns (Stewart et al., 1997). As such, gender differences in the pattern of psychophysiological stress responses may underlie why relationships differed across the two current studies. Analysis of the current studies revealed gender differences in HR reactivity, but only for the competitive stress task (see supplementary Table 4.1.). As such, research is warranted to further explore factors that are associated with these gender differences, and also in individuals with clinical anxiety who consistently interpret their anxiety symptoms as debilitative, compared to healthy individuals.

It must be noted that males reported facilitative cognitive and somatic anxiety interpretations during the competition task compared to debilitative interpretations by females. Several reasons could underlie these gender discrepancies. First, males may have more experiencing playing competitive car racing tasks, and thus have more confidence in their ability. Research using competitive stress has shown whilst performance does not differ across genders, males perceive they performed better (Veldhuijzen Van Zanten et al., 2004). Together with research highlighting confidence as a key moderator of anxiety interpretation, this may explain the facilitative anxiety reported in males (Hanton, Mellalieu, & Hall, 2004).

However, it must be noted in the current study, gender differences in anxiety interpretation were not significant.

It is interesting to observe that in study 1, males' actual HR was correlated with perceived HR change, whereas there was dissociation in study 2 in females. One possible explanation is that, whilst in study 2 a negative directionality was added to the scale, across the three tasks only 3 individuals reported a perceived decrease in HR. Given perceived increases in HR could only be reflected on 3 points of the scale, it is possible that individuals were able to detect subtle differences in HR increases, but that the 3 points of the scale were not specific enough to tease out these subtle differences between participants. Therefore, a correlation was not evident due to the spread of scores being too constrained. The current results highlight some interesting covariation between anxiety states and perceived physiological activity, but future work should refine and standardise the perceived HR measurement. We recommended that future studies should use a bi-directional scale but extend the scale to reduce issues regarding score constraint, or use actual HR beats per minute as anchors.

A limitation of the present body of work is that the single item measures of perceived HR change were developed specifically for these two studies, and as such are not a validated questionnaire to assess subjective changes in perceived HR. As such, comparisons across studies must be interpreted with caution due to the altered measurement method. Second, the measures of anxiety are single item questions which can reduce reliability, however the IAMS questionnaire has been validated for assessing cognitive and somatic anxiety against longer scales which produce valid and reliable measures of cognitive and somatic anxiety intensity and interpretation, particularly when in close proximity to a stressful event (Thomas et al., 2002). Third, utilising laboratory stressors to evoke psychological stress may induce smaller

responses compared to real life-stressors. However, by implementing the current study methodology, aggregating three types of stress tasks to produce an overall stress reactivity score, this creates a more valid and reliable methodology to assess stress responses and improve lab-to-life generalisability (Kamarck, Debski, & Manuck, 2000). Fourth, the current two separate samples were relatively homogenous. While this increases the internal validity of the results, future work should explore the current research question in clinical mixed gender populations so that results can be generalised, and the influence of gender can be examined.

In conclusion, the present two studies using both healthy male and female samples demonstrated that subjective perceptions of HR change during acute psychological stress are consistently associated with anxiety. The data extend previous literature by demonstrating subtle differences in the contribution of HR perceptions towards experiencing cognitive and somatic anxiety during stress. As hypothesised individuals, who perceived greater increases in HR change, tend to display greater levels of somatic anxiety during acute psychological stress. In addition, the work was the first to demonstrate initial evidence that HR reactivity during stress is associated with perceiving anxiety as more debilitative. The results have important implications for stress and emotional regulation. Altering perceptions of HR responses experienced during stress, may reduce the anxiety and emotional distress experienced. Second, utilising strategies to cope with the physiological HR symptoms during stress may help individuals to interpret their anxiety as less debilitating.

**Supplementary Table 4.1.**
Mean (SD) heart rate reactivity and anxiety variables for males (study 1) and females (study 2).

| | Heart rate reactivity (bpm) | |
|---|---|---|
| | Male | Female |
| Competition Task | 12.92 (10.44) | 9.40 (8.21)* |
| PASAT Task | 11.90 (8.19) | 9.73 (7.05) |
| Puzzle Task | 3.86 (4.19 | 3.38 (5.20) |
| | Cognitive Anxiety Intensity | |
| | Male | Female |
| Competition Task | 3.42 (1.54) | 3.60 (1.50) |
| PASAT Task | 5.26 (1.51) | 5.33 (1.18) |
| Puzzle Task | 3.81 (1.56) | 3.78 (1.62) |
| | Somatic Anxiety Intensity | |
| | Male | Female |
| Competition Task | 3.34 (1.56) | 3.70 (1.61) |
| PASAT Task | 4.24 (1.71) | 4.51 (1.62) |
| Puzzle Task | 2.79 (1.39) | 2.85 (1.41) |
| | Cognitive Anxiety Interpretation | |
| | Male | Female |
| Competition Task | 0.21 (1.34) | -0.21 (1.31) |
| PASAT Task | -1.21 (1.44) | -1.12 (1.50) |
| Puzzle Task | -0.22 (1.43) | -0.19 (1.28) |
| | Somatic Anxiety Interpretation | |
| | Male | Female |
| Competition Task | 0.02 (1.32) | -0.04 (1.29) |
| PASAT Task | -0.80 (1.25) | -0.96 (1.41) |
| Puzzle Task | -0.28 (1.22) | -0.13 (1.16) |

*Significantly different at $p < .05$ from male sample.

# CHAPTER 5

## ULCERATIVE COLITIS AND STRESS: PSYCHOPHYSIOLOGICAL RESPONSES TO ACUTE PSYCHOLOGICAL STRESS AND THEIR ASSOCIATION WITH ULCERATIVE COLITIS SYMPTOM BURDEN

This chapter is currently in preparation for submission:

Trotman, G. P., Kane, K., Cooney, R., Williams, S. E., & Veldhuijzen van Zanten, J. J. C. S. (in preparation). Ulcerative colitis and stress: Psychophysiological responses to acute psychological stress and their association with ulcerative colitis symptom burden.

## Chapter 5 Preface

Chapter 4 revealed that greater perceptions of heart rate change during acute psychological stress were associated with greater anxiety responses, whereas actual heart rate reactivity was unrelated to anxiety responses. These findings suggest that during acute psychological stress, it is people's perceptions of how their physiology changes that is related to how anxious they feel. In addition, the findings highlight that there is dissociation between physiological and psychological stress responses. It would be interesting to explore how physiological and psychological responses contribute to the experience of disease burden in a clinical population which is thought to be influenced by stress. One such clinical population where disease activity is proposed to be associated with stress is ulcerative colitis. Ulcerative colitis is a chronic inflammatory disease, where stress is proposed to influence disease activity by altering autonomic and inflammatory activity. Consequently, Chapter 5 explored whether patients with ulcerative colitis experience altered stress responses (including cardiovascular, inflammatory, and psychological stress responses) compared to healthy control participants. Further, Chapter 5 examined the relationship between physiological and psychological stress responses with ulcerative colitis disease burden. Given the dissociations reported in Chapter 4, it was of interest to explore whether physiological or psychological responses more strongly associated with reported disease burden.

## Abstract

Stress is proposed to exacerbate ulcerative colitis (UC), yet the exact mechanisms relating stress and UC disease activity remains unclear. This study examined whether UC patients demonstrate altered physiological or psychological responses to stress and whether stress responses relate to UC symptom burden. Twenty-nine patients with UC (*n* = 15 male) and ten healthy control volunteers (n = 5 male) completed a single laboratory session including, 10-min baseline period, 8-min standardised psychological stress task (paced auditory serial addition task), followed by a 60-min post-stress rest period. Heart rate, blood pressure, heart rate variability, pre-ejection period, and interleukin-6 were assessed during all periods. Psychological measures including anxiety, primary and secondary appraisals, affect, and perceived heart rate change were assessed immediately following the stress. Patients with UC reported current symptom burden and a sub-sample of UC patients (n = 11) provided a stool sample for measurement of faecal calprotectin on the morning of testing, and the following morning. No group differences for cardiovascular activity were observed, however UC patients demonstrated lower vagal activity at rest and during stress. Acute psychological stress induced increases in IL-6 in both groups, with UC patients demonstrating significantly greater IL-6 at rest, during stress and post-stress. Increases in FCAL were reported the day following stress. With the exception of perceived control, no group differences were observed for psychological responses. No associations between physiological stress responses and UC symptom burden were evident, but consistent positive associations between UC symptom burden and stress induced negative psychological states (perceived threat, negative affect, perceived heart rate change and perceived stress) were revealed. Psychological factors may play an important role linking stress and UC symptom burden, yet physiological responses to stress appear unrelated.

## Introduction

Ulcerative colitis (UC) is a debilitating chronic inflammatory gastrointestinal disorder, and is the most common type of inflammatory bowel disease (IBD), with prevalence in European countries reported as 505 per 100,000 (Ng et al., 2017). Characterised by chronic relapsing clinical symptoms including bloody diarrhoea, abdominal pain, and fatigue, UC has a significant impact on overall quality of life. The specific mechanisms resulting in disease relapse are not fully understood, thus, examining factors that contribute to symptom exacerbation can have significant clinical impact.

Psychological stress has been suggested to contribute to disease exacerbation in UC. A review of 18 prospective studies identified 13 studies demonstrating a significant association between higher levels of stress and adverse UC and other IBD outcomes (Camara et al., 2009). Yet, significant methodological limitations were apparent in the reviewed literature such as not differentiating between UC and other IBD disease groups such as those with Crohn's disease (CD), using an assortment of stress measurement tools, not comprehensively examining the role of stress appraisals and perceptions of stress, as well as not assessing markers of inflammation (i.e., an objective marker of disease activity).

More recently, large-scale studies have investigated the relationships between perceived stress, self-report symptom activity and inflammation as measured with faecal calprotectin (FCAL; a protein that is released in stool which correlates with degree of inflammation in the bowel lining and is used clinically as a non-invasive marker of disease activity) (Sexton et al., 2017; Targownik et al., 2015). Perceived stress was associated with greater self-reported IBD symptoms in both UC and CD patients using cross-sectional (Targownik et al., 2015) as well as prospective longitudinal analyses (Sexton et al., 2017). However, no associations were evident between perceived stress and intestinal inflammation (Sexton et al., 2017; Targownik

et al., 2015). Furthermore, other psychological factors seem to play an influential role in the course of IBD (Bernstein, 2016; Mikocka-Walus, Pittet, Rossel, von Kanel, & Swiss, 2016). For example, patients with IBD have increased rates of anxiety and depression (Sajadinejad et al., 2012), which are strongly associated with the recurrence of symptoms (Mikocka-Walus et al., 2016). Additionally, negative affect along with perceived stress was associated with greater IBD symptoms in a 12-month prospective study (Bernstein et al., 2010). Given the associations between perceived stress, anxiety, negative affect and IBD symptoms but lack of associations between perceived stress and intestinal inflammation (Sexton et al., 2017; Targownik et al., 2015), psychological factors may specifically play a prominent role linking stress and reported UC disease symptoms. A greater understanding of how stress influences the course of IBD is needed, in order to provide appropriate information to patients to reduce the risk of disease exacerbation.

It is well known that exposure to acute psychological stress perturbs cardiovascular function through alterations in sympathetic and parasympathetic activity (Brindle et al., 2014), upregulates pro-inflammatory cytokines (Marsland et al., 2017), and induces negative psychological states including anxiety and negative mood (Trotman et al., 2018). Assessing reactions to stress-evoking situations allows an investigation into whether the physiological or psychological responses are a pathway linking stress to UC disease activity. While experiencing acute psychological stress, many physiological systems are activated, including involving cognitive brain structures, the autonomic nervous system, the hypothalamic-pituitary-adrenal axis (HPA) and enteric nervous system (Brzozowski et al., 2016; Osadchiy, Martin, & Mayer, 2019). This linking of psychological and physiological responses centrally and peripherally is labelled the brain-gut axis and highlights how psychological stress can influence gut homeostasis (Bonaz et al., 2018; Osadchiy et al., 2019). However, evidence

implicating stress and the brain-gut axis in UC has primarily been based on animal models. Stress could augment UC symptoms and patient burden, yet there are few studies that have investigated whether patients with UC demonstrate an altered stress response or whether stress responses relate to disease symptom burden.

To our knowledge, only one human study has directly explored the hypothesis that UC patients exhibit an altered stress response (Mawdsley et al., 2006), with one further study using a mixed UC and CD sample (Kuroki et al., 2011). Mawdsley et al. (2006) demonstrated no stress-induced changes in serum IL-6 or IL-13 in the IBD or control group to a 50-min IQ test. Further, in a mixed IBD sample of UC and CD participants, baseline differences in IL-6 were observed and IL-6 increased during stress in IBD patients, but not in controls. No increases during stress or differences between groups in any other inflammatory (TNF- α, IL-1ß) or HPA-axis markers during stress were reported (Kuroki et al., 2011). Importantly, the above studies may have missed important variance in inflammatory cytokine responses in the period following stress exposure. That is, stressor-induced cytokine activation peaks between 31 and 90 minutes post-stress (Marsland et al., 2017), yet studies with UC patients have not measured cytokine activity in this window. In addition, Mawdsley et al. (2006) assessed cardiovascular activity during stress (pulse rate, systolic and diastolic blood pressure), demonstrating stress-induced increases but no differences between UC and controls in response to stress. However, no study has investigated in UC the autonomic responses to stress, which are important given the associations between the parasympathetic system and inflammation (Bonaz & Bernstein, 2013). Further, no study has directly investigated whether UC patients experience greater negative psychological responses to acute psychological stress.

Addressing the limitations outlined in the literature (Camara et al., 2009; Keefer, Keshavarzian, & Mutlu, 2008), the current study recruited UC patients as well as a control comparison group. To gain a more comprehensive understanding, based on the transactional model of stress and coping framework (Lazarus & Folkman, 1984), biopsychosocial variables were measured, including psychological variables and objective physiological measures. The aims were to 1) examine whether patients with UC exhibit altered cardiovascular, autonomic, inflammatory or psychological responses to acute stress compared to healthy controls, and 2) examine the associations of UC symptom burden with cardiovascular, autonomic, inflammatory and psychological responses to acute psychological stress.

## Method

### Participants

Twenty nine patients with UC[1] and ten healthy control volunteers completed the study. Ulcerative colitis patients were recruited from University Hospital Birmingham NHS Trust (Queen Elizabeth Hospital Birmingham) and UC was verified with clinical, endoscopic and histological criteria. Healthy control participants were recruited from University staff, students, and the local community. Exclusion criteria included history of cardiovascular, kidney, or liver disease, autonomic dysfunction, currently pregnant, not fluent in English, or if changed medication in the previous 8-weeks. The study obtained ethical approval from the Health Research Authority Committee, and all participants provided informed consent prior to participation.

[1] One patient was reported to take propranolol medication (beta-blocker). Subsequent analyses on cardiovascular and inflammatory data were conducted with and without this patient and the results remain unchanged. As a result the patient remained in the main analysis.

## Cardiovascular and Autonomic Measures

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed using an oscillatory blood pressure monitor (Omron HEM-705CP) with a cuff attached over the participant's brachial artery. Using 7-electrodes, the electrocardiogram (ECG) and impedance cardiogram (ICG) were continuously assessed with the Ambulatory Monitoring System VU-AMS5fs (TD-FPP, Vrije Universiteit, Amsterdam, The Netherlands; de Geus et al., 1995). Automated inter-beat interval time series detection in the VU-DAMS software provided measures of heart rate (HR; bpm) and heart rate variability (HRV) measured as the square root of mean squared differences in R-R intervals (RMSSD; ms). Following inspection of the ICG and manual correction where necessary, pre-ejection period (PEP; ms) was derived following ICG scoring guidelines (Nederend, ten Harkel, Blom, Bernston, & de Geus, 2017; Sherwood, Allen, et al., 1990). Pre-ejection period is an indirect marker of sympathetic activity (Cacioppo et al., 1994), and RMSSD is an indirect marker of parasympathetic activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Thomas, Claassen, Becker, & Viljoen, 2019).

## Inflammatory Markers

**Serological inflammation.** A 20G cannula was inserted into the antecubital vein in the participant's arm. At each time point, 6 ml blood was taken into a vacutainer containing K2EDTA (Becton–Dickinson, UK) and stored on ice. Samples were subsequently centrifuged at 1500 × g for 10 min at 4 ºC and plasma aliquots were stored at -80 ºC until further analysis. Levels of interleukin-6 (IL-6) were analysed in duplicate using high sensitivity ELISAs following manufacturer guidelines, with intra-assay variations in the current study at 5.01%.

**Faecal calprotectin.** A sub-sample of UC patients were provided with stool kits, and asked to provide a stool sample on the day of the lab-visit, and a second sample on the subsequent day ($n$ = 11). Stool samples were kept at a temperature of -20 ºC prior to, and 4 ºC following extraction. Measures of FCAL was assessed with a particle-enhanced turbidimetric immunoassay (Bühlmann, Schönenbuch, Switzerland), with a control solution used prior to and following each assay.

## Trait Psychological Questionnaires

**Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983).** The PSS assesses recent perceived life stress, and has high internal consistency with the Cronbach's alpha at .85 (Cohen et al., 1983). Ten-items are answered on a 0 (never) to 4 (very often) scale, regarding how often participants felt a certain way in the last month (e.g., "How often have you felt difficulties were piling up so high that you could not overcome them?"). The scores on all questions are summed with a higher score reflecting greater perceived stress.

**Perceived Stress Questionnaire (Levenstein et al., 1993).** The PSQ assesses long-term stressful life events. Thirty-items are answered on a 1 (almost never) to 4 (usually) scale, regarding how often each item applied to the participant in the previous year (e.g., "your problems seem to be piling up' and "you have trouble relaxing"). The PSQ has been validated and demonstrates test–retest reliability of .82 (Levenstein et al., 1993). The PSQ items are summed, and a PSQ index is calculated (raw score-30/90) yielding a score between 0 and 1, with a greater score representing greater experienced stress.

**Anxiety and depression.** The Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983) which has been validated in non-psychiatric settings (Bjelland,

Dahl, Haug, & Neckelmann, 2002) assessed anxiety and depression. Fourteen items are answered on a four item scale from 0 – 3, regarding how the participant has been feeling in the previous week. Following item reversal, all items on each subscale for anxiety (7-items; e.g., "I feel tense or wound up") and depression (7-items; e.g., "I feel as if I am slowed down") are summed to obtain separate scores for anxiety and depression. Scores for each subscale range from 0 – 21, with greater scores representing greater symptoms.

**UC Symptom Burden (UC patients only)**

**The IBD disk (Ghosh et al., 2017).** The IBD disk is a ten-item self-assessment of IBD-disability and computes an overall disease-related score of symptom burden. Ten-items are scored on a scale from 0 (absolutely disagree) to 10 (absolutely agree), regarding the experience of each item in the previous week. The IBD disk assesses a range of health related functions including bodily (e.g., "I have had aches or pains in my stomach or abdomen"), social (e.g., I have had difficulty with personal relationships and/or difficulty participating in the community") and psychological (e.g., "I have felt sad, low or depressed, and/or worried or anxious") related IBD-domains. The IBD disk was developed from the IBD-disability index which assesses IBD health, functioning and disability (Peyrin-Biroulet et al., 2012) and has been validated for use in clinical trials and research studies (Gower-Rousseau et al., 2017). A greater total score between 0 and 100 reflects greater total UC symptom burden.

**Acute Stress Task Questionnaires**

The following items were all completed immediately following the stress task, asking participants how they felt during the task.

**Task engagement.** Separate 7-point Likert-scale items assessed the perceived task difficulty and engagement ranging from 0 (not at all) to 6 (extremely) and the extent participants were trying from 0 (did not try at all) to 6 (tried throughout the entire task).

**Primary appraisals.** Primary appraisals represent the personal relevance or threat appraised by individual (Lazarus & Folkman, 1984). To evaluate this, separate 7-point Likert scales assessed how demanding (0 = not at all, to 6 = extremely) and stressful (-3 = large decrease in stress level, to +3 = large increase in stress level) participants found the task. Perceived threat was assessed with three items (e.g., "I view the task as a threat"), adapted from McGregor and Elliot (2002), which have been used previously to assess stress-induced threat (Trotman et al., 2018). The three items are scored on a scale from 0 (not at all) to 6 (very true) and averaged. The Cronbach's alpha was .95 in the current sample.

**Secondary appraisals.** Secondary appraisals represent the perceived coping ability and controllability for a given situation (Lazarus & Folkman, 1984). To evaluate this, perceived ability to cope and perceived control were assessed with separate 7-point Likert scales, from 0 (not at all) to 6 (completely) which are commonly used in the stress literature (Moore, Wilson, Vine, Coussens, & Freeman, 2013; Trotman et al., 2018)

**Anxiety.** The Immediate Anxiety Measures Questionnaire (IAMS; Thomas et al., 2002) assesses cognitive and somatic anxiety intensity, from 1 (not at all) to 7 (extremely), and whether symptoms were perceived as being positive or negative, from -3 (very debilitative/negative) to +3 (very facilitative/positive) . The IAMS has been validated against multi-item state anxiety questionnaires and provides reliable anxiety scores, particularly in close proximity to a stressful event (Thomas et al., 2002).

**Affective state.** Affect during the task was assessed with the Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988). Twenty items are answered on a 1 (very slightly or not at all) to 5 (extremely) scale. The 10 positive items (e.g., "enthusiastic") and 10 negative items (e.g., "distressed") are separately summed so scores range from 10 – 50 for each subscale. The PANAS demonstrates satisfactory reliability, test-retest reliability and has been validated in community and clinical samples (Crawford & Henry, 2004), with Cronbach alphas in the current sample at .89 and .84 for positive and negative scales.

**Perceived HR change.** A single item scale from -6 (I experienced an extreme decrease in HR) to +6 (I experienced an extreme increase in HR) assessed how much participants perceived their HR changed during the task. The scale has been used in previous work examining perceived HR changes during acute psychological stress (Trotman et al., 2019).

**Acute Psychological Stress Task**

Participants completed an 8-min version of the Paced Auditory Serial Addition task (PASAT; Gronwall, 1977). The PASAT reliably perturbs the cardiovascular system (Ring et al., 2002; Veldhuijzen Van Zanten et al., 2004) and has demonstrated strong test-retest reliability (Ginty et al., 2013). A series of single-digit numbers were presented through audio speakers, and participants were asked to add sequential numbers together. Participants must verbalise their answers whilst remembering the previous number to add it to the next number presented. To increase stress, the time between numbers presented decreased every two minutes from 2.4s to 2.0s to 1.6s and 1.2s. Participants were videotaped, which was displayed on a television directly in front of them, and they were informed body language experts would analyse the video to assess anxiety levels; in reality, no such body language analysis took place. The experimenter sat opposite the participant marking their performance and a brief

loud aversive noise was presented at standardised intervals to indicate an incorrect response, regardless of the answer, which has been shown to increase the stress experienced (Veldhuijzen Van Zanten et al., 2004). Participants were informed they began with 1000 points and lost five points for every incorrect or missed answer and a leader board was displayed with faux results from participants in the study.

**Procedure**

Participants were sent a survey questionnaire pack to complete prior to their laboratory session. A sub-sample of the UC group provided a stool sample on the day of the lab-visit, and a second sample on the subsequent day ($n$ = 11). Upon arrival at the laboratory, participants provided the stool sample and had their height and weight measured to calculate body mass index (BMI; $kg/m^2$). Seven spot electrodes were attached for ECG and ICG recording, a cannula was inserted for blood sampling, and a blood pressure cuff was attached to the contralateral arm. Participants began a 10-min adaptation period, followed by a 10-min resting baseline where a nature documentary DVD was watched while participants remained quiet. Blood pressure was measured every other minute during baseline, with ECG and ICG data analysed during these minutes, and a blood sample was taken immediately following the baseline period. Subsequently, audio-instructions were provided regarding the stress task, and a 15-second practice task was completed to ensure participants understood the task. Participants then completed the 8-min stress task with blood pressure measurements taken every other minute and ECG and ICG analysed for these minutes. A blood sample was taken immediately after the stress task, followed by completing the post-task questionnaire pack. Finally, participants completed a 60-minute post-task rest period with blood samples obtained

in minutes 30 and 60, before all equipment was removed, and participants were thanked and debriefed.

**Data Reduction and Statistical Analysis**

Cardiovascular measures during baseline (mins 2, 4, 6, 8, 10) and stress task (mins 2, 4, 6, 8) periods, and reactivity (stress task minus baseline) was computed. Due to a significant positive skew in IL-6 (Shapiro-Wilk test, all *p's* < .005), values were log transformed for analyses. Following log-transformation, IL-6 was normally distributed. FCAL and psychological responses to stress remained non-normally distributed even after log transformation, therefore non-parametric tests on the raw data were used to analyse these variables. Data was inspected, and outliers exceeding 3 standard deviations were removed from the respective analyses (HR; *n = 1,* IL-6; *n = 1,* perceived HR; *n* = 1*).* Missing data due to equipment malfunction is reflected in slight variations in degrees of freedom for the respective analyses. Independent sample *t*-tests compared UC and control group's participant characteristics and trait psychological measures. Separate Mann–Whitney U tests examined whether UC patients and controls differed in reported stress task evaluations. To explore physiological stress responses, a 2 group (UC, control) × 2 time (baseline, stress) MANOVA was run for cardiovascular variables, and a 2 group (UC, control) × 4 time (baseline, stress, 30mins post-task, 60mins post-task) ANOVA was run for IL-6. A Wilcoxon signed-rank test explored whether FCAL changed from day 1 to day 2. Mann–Whitney U tests were run to compare UC and control participants' acute stress psychological responses. In UC patients, to explore the relationship between UC symptom burden and cardiovascular, inflammatory and psychological variables, IBD disk items were summed and Pearson's correlations with UC IBD disk summed scores were run with physiological variables, and Spearman's rank

correlations were run with psychological variables. Greenhouse Geisser was reported for all ANOVAs and alpha was set at <.05 for all analyses.

## Results

### Participant Characteristics

Demographics, trait psychological data and clinical characteristics are presented in Table 5.1. No differences in age, gender or BMI were observed between UC and control groups (*p*'s >.05). Further, no differences in trait psychological data including anxiety, depression, short-term or long-term perceived stress were evident (*p's* >.05).

### Stress Manipulation Checks

Participants reported a moderate level of stress, a large degree of difficulty, as well as being highly engaged. No differences between groups were observed (*p's* > .05) suggesting the task induced an equal amount of stress across both groups (Table 5.1).

### Cardiovascular Responses to Acute Psychological Stress

A 2 group (UC, controls) × 2 time (baseline, stress) MANOVA for cardiovascular variables revealed a multivariate time effect, Pillai's trace = .960, $F(5,23) = 110.41$, $p < .001$, $\eta p^2 = .960$, and time × group interaction effect, Pillai's trace = .421, $F(5,23) = 3.35$, $p = .02$, $\eta p^2 = .421$, but no significant group effect, Pillai's trace = .345, $F(5,23) = 2.42$, $p = .067$, $\eta p^2 = .345$. Follow-up univariate ANOVAs revealed time effects for HR, PEP, HRV, SBP and DBP, as well as a time × group interaction for HRV only (Table 5.2). Post-hoc analyses showed HR, SBP and DBP significantly increased and PEP decreased during stress from baseline in both UC and control participants. Compared to controls, UC patients demonstrated

significantly lower HRV during baseline ($p$ <.001) and stress ($p$ = .013), and HRV significantly decreased during stress in the control participants only.

**Table 5.1.** Mean (SD) values of participant characteristics, trait psychological measures and stress task manipulation checks.

| | Ulcerative Colitis | Control |
|---|---|---|
| Participant Characteristics | Mean (SD) | Mean (SD) |
| Age (years) | 42.72 (15.77) | 35.20 (16.19) |
| Gender (male/female) | 15 / 14 | 5 / 5 |
| BMI (kg/m$^2$) | 26.83 (4.24) | 24.69 (2.39) |
| IBD disk SUM score | 41.30 (22.18) | - |
| Trait Psychological Measures | | |
| HADS Anxiety | 7.41 (4.08) | 6.40 (4.55) |
| HADS Depression | 4.48 (3.26) | 2.60 (4.65) |
| Perceived Stress Scale | 18.41 (6.06) | 15.50 (7.56) |
| Perceived Stress Questionnaire | 0.43 (0.18) | 0.37 (0.19) |
| Stress Task Manipulation Checks | | |
| Perceived Difficulty | 5.2 (1.0) | 5.2 (0.8) |
| Perceived Stressfulness | 1.8 (0.8) | 1.8 (0.6) |
| Extent Engaged | 4.9 (1.2) | 4.7 (1.1) |
| Duration of Disease (years) | 9.59 (10.2) | - |
| Current Ulcerative Colitis Medication | | - |
| Mesalazine / Salofalk | 23 (76%) | - |
| Adalimimumab | 5 (17%) | - |
| Infliximab | 2 (7%) | - |
| Salazopyrine | 3 (10%) | - |
| Azathioprine/ Mercaptopurine | 4 (14%) | - |
| Emtricitabine/ Tenofovir | 1 (3%) | - |
| Type of UC | | |
| Total | 10 (34%) | - |
| Left-sided | 10 (34%) | - |
| Proctitis | 5 (17%) | - |
| Unknown | 2 (7%) | - |

*Note: HADS-Anxiety (0-21); HADS-Depression (0-21); PSS (0-40); PSQ (0-1); IBD disk SUM score (0-100); Perceived difficulty (0-6); Stressfulness (-3 - +3); Engaged (0-6). Some patients were on a combination of medications for the treatment of UC.*

**Table 5.2.** Mean (*SD*) values of cardiovascular variables during baseline and stress.

| | Ulcerative Colitis | | Control | | | | |
|---|---|---|---|---|---|---|---|
| | Mean (*SD*) | | Mean (*SD*) | | Time effect | Group effect | Interaction |
| | Baseline | Stress | Baseline | Stress | *p* | *p* | *p* |
| Heart rate (bpm) | 70.83 (10.52) | 74.93 (9.91) | 67.22 (7.18) | 73.45 (10.29) | **<.001** | .92 | .83 |
| Systolic blood pressure (mmHg) | 119.76 (13.83) | 143.44 (22.99) | 115.47 (11.19) | 137.12 (19.65) | **<.001** | .15 | .15 |
| Diastolic blood pressure (mmHg) | 77.31 (11.75) | 92.27 (15.15) | 75.92 (7.01) | 88.10 (11.37) | **<.001** | .41 | .31 |
| Heart rate variability (ms) | 27.11 (19.08) | 23.92 (12.79) | 51.29 (19.13) | 38.80 (16.70) | **.003** | **.001** | **.009** |
| Pre-ejection period (ms) | 123.48 (17.15) | 114.81 (18.20) | 127.33 (14.51) | 123.97 (22.02) | **<.001** | .82 | .53 |

*Note:* Results are reported for repeated measures MANOVA univariate tests.

**Il-6 Responses to Acute Psychological Stress**

A 2 group (UC, controls) × 4 time (baseline, stress, 30mins post task, 60mins post task) ANOVA revealed a significant group effect, F(1,21) = 5.47, $p$ = .029, $\eta p^2$ = .207, time effect, F(1,31) = 16.08, p <.001, ηp2 = .434, but no significant interaction, F(1,31) = 0.14, $p$ = .801, $\eta p^2$ = .007, for IL-6. Post-hoc analyses revealed IL-6 remained unchanged during stress compared to baseline, but increased at 30mins post task, and further increased at 60mins post task, in both groups. IL-6 was significantly greater in UC patients at all time points (Figure 5.1).

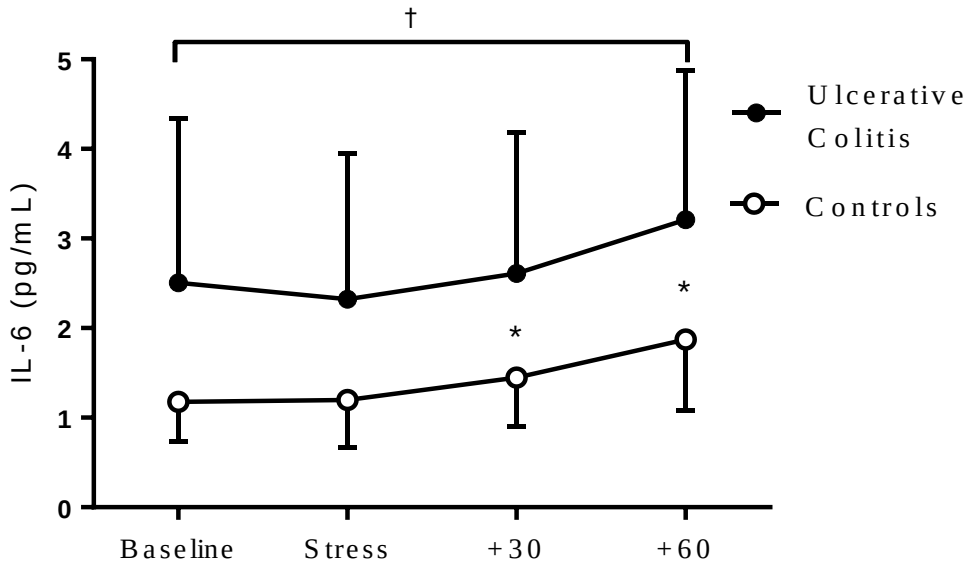


**Figure 5.1**. Mean (SD) Interleukin-6 (IL-6) values during baseline, stress, 30-mins and 60-mins post-stress. *Significant time effect, p <.05. †Significant group effect, p <.05

**Faecal Calprotectin Responses to Acute Psychological Stress**

A Wilcoxon signed-rank test for 9 UC patients where both samples were available revealed a significant time effect (Z = -2.201, $p$ = .028), demonstrating FCAL increased from

the morning of acute stress testing (130.44 ± 171.89 pg/ml), compared to the subsequent morning (190.00 ± 214.85 pg/ml).

**Psychological Responses to Acute Psychological Stress**

Mann-Whitney U tests found no significant group differences for primary appraisals, including perceived demands and perceived threat (*p's* > .05; Table 5.3). For secondary appraisals, no difference was found for perceived coping ability (*p* >.05), however UC patients reported significantly lower perceived control during stress (U = 74, *p* = .020). No significant group differences were observed for positive and negative affect, cognitive and somatic anxiety intensity and perceived interpretation of symptoms, or perceived HR change during stress (*p's* > .05; Table 5.3).

**Associations between Stress Task Responses and UC Burden**

**Physiological responses and UC burden.** No associations were observed between UC symptom burden and stressor-evoked cardiovascular reactivity, IL-6 reactivity, or FCAL reactivity (*p's* > .05; Supplementary Table 5.1).

**Psychological responses and UC burden.** All correlations are presented in Table 5.4. Significant positive associations were found between total UC symptom burden and primary appraisal perceived threat ($r(28) = .423$, $p = .028$). No associations between UC symptom burden and perceived demand, or secondary appraisals perceived control or coping ability were evident (*p's* > .05). Associations were observed between perceived threat and IBD disk items including emotions and body image as well as perceived coping and IBD disk item sexual function (*p's* <.05). Total UC symptom burden was significantly and positively

associated with stress-induced negative affect, ($r(28) = .636$, $p < .001$) and perceived HR change during stress, ($r(26) = .538$, $p = .006$).

**Table 5.3.** Mean (SD) psychological responses during stress.

| Psychological Responses | Ulcerative Colitis | Control |
|---|---|---|
| Primary Appraisals | | |
| Perceived Demands | 5.2 (0.8) | 5.0 (0.8) |
| Perceived Threat | 2.2 (2.0) | 1.3 (1.5) |
| Secondary Appraisals | | |
| Perceived Coping | 2.6 (1.6) | 2.3 (1.6) |
| Perceived Control | 1.9 (1.5) | 3.6 (1.9)* |
| Anxiety | | |
| Cognitive Anxiety Intensity | 5.3 (1.7) | 4.8 (1.8) |
| Somatic Anxiety Intensity | 4.4 (1.6) | 3.5 (1.9) |
| Cognitive Anxiety Interpretation | -1.2 (1.7) | -0.5 (1.6) |
| Somatic Anxiety Interpretation | -0.9 (1.4) | -0.3 (1.0) |
| Affect | | |
| Positive Affect | 27.0 (8.6) | 25.2 (4.6) |
| Negative Affect | 19.6 (6.6) | 18.3 (6.7) |
| Heart Rate Perceptions | | |
| Perceived Change in heart rate | 2.9 (1.6) | 2.3 (1.5) |

*Significantly different from UC patients, $p < .05$.

*Note: Perceived demands, threat, coping & control (0-6); cognitive and somatic intensity (1-7); cognitive and somatic interpretation (-3 - +3); Positive and negative affect (10 – 50); Perceived change in heart rate (-6 - +6).*

**Table 5.4.** Correlation between UC symptom burden and psychological responses to acute psychological stress.

| Psychological Responses | IBD DISK SUM | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Primary Appraisals | | | | | | | | | | | |
| Perceived Demands | .22 | .23 | .01 | .15 | .12 | .10 | .18 | .27 | .11 | .18 | .14 |
| Perceived Threat | .42* | .11 | .14 | .22 | .23 | .25 | .36 | .53** | .48* | .30 | .12 |
| Secondary Appraisals | | | | | | | | | | | |
| Perceived Coping | -.18 | -.07 | -.19 | -.04 | -.18 | .05 | -.02 | -.12 | -.35 | -.43* | -.08 |
| Perceived Control | -.18 | .13 | .14 | -.08 | -.88 | -.33 | -.27 | -.22 | -.36 | -.10 | -.19 |
| Anxiety | | | | | | | | | | | |
| Cognitive Anxiety Intensity | .08 | -.08 | -.12 | .01 | .02 | .07 | .13 | .09 | .38* | .13 | .15 |
| Somatic Anxiety Intensity | .30 | .15 | -.04 | .13 | .14 | .21 | .16 | .20 | .41* | .24 | .27 |
| Cognitive Anxiety Interpretation | .05 | .12 | .24 | .15 | .10 | .06 | .03 | .08 | -.26 | -.23 | -.19 |
| Somatic Anxiety Interpretation | -.03 | .04 | .34 | -.01 | .05 | -.15 | -.13 | -.01 | -.22 | -.10 | -.14 |
| Affect | | | | | | | | | | | |
| Positive Affect | -.06 | -.12 | .12 | -.03 | -.16 | -.07 | -.12 | -.06 | -.25 | -.22 | .15 |
| Negative Affect | .64*** | .40* | .36 | .40* | .43* | .37 | .55** | .57** | .68*** | .28 | .15 |

| Psychological Responses | IBD DISK SUM | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Heart Rate Perceptions | | | | | | | | | | | |
| Perceived Change in HR | .54** | .71*** | .04 | .42* | .51** | .19 | .43* | .52** | .40** | .26 | .16 |
| Stress Task Evaluations | | | | | | | | | | | |
| Perceived Difficulty | .15 | -.01 | .02 | .04 | -.02 | .09 | .07 | .17 | .37 | .34 | .13 |
| Perceived Stressfulness | .38* | .32 | .17 | .28 | .14 | .14 | .36 | .33 | .39* | .33 | .12 |
| Extent Engaged | -.03 | -.01 | -.27 | -.01 | -.08 | -.01 | -.01 | .05 | -.16 | -.30 | .33 |

***Significant association, $p < .001$, **Significant association, $p < .01$.*Significant association, $p < .01$.

*Note:* 1. Abdominal pain 2. Regulating defecation 3. Interpersonal interactions 4. Education and work 5. Sleep 6. Energy 7. Emotions 8. Body image 9. Sexual functions 10. Joint pain

No associations between total UC symptom burden and positive affect, cognitive and somatic anxiety intensity or interpretation during stress and were evident (*p's* > .05). Notably, negative affect and perceptions of HR during stress were consistently related to nearly all items of the IBD disk (*p's* <.05). Further, cognitive and somatic anxiety intensity reported during the stress task were positively associated with IBD disk item 'body image' (*p's* <.05).

**Task evaluations and UC burden.** A significant association was demonstrated between total UC symptom burden and perceived stressfulness of the stress task ($r(28) = .381$, $p = .050$), with no associations with perceived difficulty or extent engaged (*p*'s >.05; Table 5.4). In addition, perceived stressfulness were positively associated with the 'body image' item of the IBD disk (*p* <.05).

## Discussion

This study aimed to explore detailed psychological and physiological responses to acute stress. This is the first study to show that in comparison to healthy control participants, patients with UC displayed reduced vagal activity and increased levels of inflammation at rest and during stress, and lower perceived control. UC symptom burden was not related to physiological stress responses but was related to psychological stress responses.

Although groups did not differ in cardiovascular responses to stress, UC patients displayed lower vagal activity at rest and reduced responses to stress compared to controls. Low HRV is associated with poor health outcomes including greater inflammation (Williams et al., 2019), cardiovascular disease and stroke (Thayer & Lane, 2007), and mortality (Dekker et al., 2000). These findings support previous work demonstrating lower vagal tone in UC at rest (Pellissier, Dantzer, Canini, Mathieu, & Bonaz, 2010), and extend this finding to show

lower vagal activity during stress. Further, in response to acute stress, UC patients did not demonstrate alterations in HRV suggesting there might be dysfunctional autonomic control in UC. Together, given the parasympathetic nervous system's role in inhibiting inflammatory activity via the vagus nerve (Bonaz et al., 2018), the reduced vagal activity at rest and during stress in the current study could play an important role in the pathway linking stress and symptom exacerbation in UC.

In the current study, stress induced increases in inflammation 30mins and 60mins post-stress, and at all-time points patients with UC demonstrated higher IL-6 compared to controls. Previous work in IBD has demonstrated mixed findings, with no increases in serum IL-6 and no group differences reported between UC patients and controls (Mawdsley et al., 2006). In contrast, a mixed UC/CD sample demonstrated elevated IL-6 at baseline compared to controls, and increases in IL-6 during stress in the UC/CD sample were reported (Kuroki et al., 2011). The current study is the first to show in UC increases in IL-6 at 30-mins and 60mins post-stress. This is in line with work in healthy populations demonstrating the peak window for stress-induced cytokine activation is 31-90mins post-stress (Marsland et al., 2017; Steptoe et al., 2007).

Consequently, if stress induces increases in inflammation in patients with already elevated inflammatory cytokine activity, this could potentially exacerbate disease activity in UC. The two previous studies examining acute stress responses in IBD included only patients with quiescent disease (Kuroki et al., 2011; Mawdsley et al., 2006). In the current study, eligibility was not limited to patients with quiescent disease, and thus greater inflammation at baseline and stress in our UC group, may result from patients having currently active disease. IL-6 in the current sample was 2.51pg/ml at baseline and 3.21pg/ml 60mins post-stress,

whereas Mawdsley et al. (2006) reported serum IL-6 values of 1.3pg/ml and 1.5pg/ml at baseline and 30mins after stress in UC patients. Alternatively, it could be that patients with already elevated inflammation may respond to stress with an accentuated inflammatory response. Indeed, it has been suggested in other inflammatory disease populations, that stress-induced inflammatory increases may be greater in patients with active disease (Kop et al., 2008; Veldhuijzen van Zanten, Ring, Carroll, & Kitas, 2005). This aligns with the only other study showing stress induced IL-6 increases, where IBD patients reported IL-6 values of 13.17pg/ml and 14.55pg/ml pre-stress and post-stress (Kuroki et al., 2011). Given previous associations between IL-6 and disease severity in UC have been reported (Hyams, Fitzgerald, Treem, Wyzga, & Kreutzer, 1993; Mitsuyama, Sata, & Tanikawa, 1991; Mitsuyama et al., 1995), it would be interesting to explore in future work if patients with active or inactive disease differ in the inflammatory response to psychological stress.

The sub-sample of UC patients who provided stool samples demonstrated significant increases in FCAL on the day after the stress task compared to the stool sample provided before the stress task. These increased levels of FCAL could be a consequence of stress altering intestinal mucosa permeability through the brain-gut axis inducing an increase in inflammation (Bonaz & Bernstein, 2013; Brzozowski et al., 2016; Michielan & D'Inca, 2015). Indeed, FCAL has been associated with greater gut permeability in IBD (Roseth, Schmidt, & Fagerhol, 1999; Tibble et al., 2000), and in healthy participants acute psychological stress can increase intestinal permeability, which is related to systemic IL-6 upregulation (Linninge et al., 2018). While the exact mechanisms are currently not fully understood, these preliminary findings suggest that stress-induced increases in inflammation could result in the exacerbation of UC.

The present study is the first to show that in response to a controlled laboratory stress task, UC patients reported significantly lower perceived control, which has been associated with greater perceived life stress as well as poorer psychological well-being (Rosenbaum, White, & Gervino, 2012; Schilling & Diehl, 2018). Given that the ability to cope and consequent behavioural adjustments determine the impact of stress on the body (Lazarus & Folkman, 1984), UC patients who have lower perceived control during stress might engage in negative coping behaviours, resulting in stress having a more detrimental impact. No other differences emerged between UC patients and control participants in the psychological responses experienced during acute stress, including anxiety, positive and negative affect, and perceived change in HR. These results are perhaps surprising given that literature demonstrates greater prevalence of trait anxiety and depression in IBD populations (Walker et al., 2008), however the severity of disease may moderate these differences (Hauser, Janke, Klump, & Hinz, 2011). Given that in the current study no group differences were evident for trait anxiety, depression and perceived stress, it could be that the current UC sample were in better psychological health compared to previous literature and future work should explore whether psychological responses to stress differ between patients in remission and with active disease.

No associations were evident between UC symptom burden with cardiovascular reactivity, IL-6 reactivity, or FCAL reactivity. While this may appear somewhat surprising given that bi-directional relationships occur between psychological factors and the gut through autonomic and immune responses (Bonaz et al., 2018; Brzozowski et al., 2016), prospective studies have reported relationships between perceived stress and self-reported UC symptoms in the absence of associations with objective FCAL inflammation (Sexton et al., 2017; Targownik et al., 2015). These prospective studies combined with the present study

suggest that the stress-symptom burden relationship may be independent of physiological mechanisms.

To our knowledge, the current study is the first to demonstrate an association between UC symptom burden and perceived stress reported during a controlled acute stress task. This adds to work demonstrating perceived life stress (in the previous months) as the strongest predictor of disease course compared to other psychological variables (Bernstein et al., 2010). In addition the current study demonstrates that key stress appraisals and mood states involved in theories of stress and coping including perceived threat, negative affect, and perceptions of HR change, are related to greater UC symptom burden. Prospective studies have demonstrated general negative affect to predict increased risk of experiencing a flare (Bernstein et al., 2010), with affective state purportedly influencing IBD disease activity through the brain-gut axis, consequently altering gut symptoms (Pellissier et al., 2010; Pellissier et al., 2014). Together, it could be that in UC, experiencing stressful situations as more threatening and with greater negative affect impacts UC symptoms through altering activity of the brain-gut-axis. However, the lack of relationships between HRV, UC burden, and affective state and appraisals in the current study outlines more work is needed to fully understand this interaction.

The relationship between perceived change in HR and UC symptom burden is particularly interesting, given the lack of associations between UC symptom burden and physiological stress markers including actual HR reactivity. The perceived physiological impact of stress may be more important than the actual biological stress responses for influencing UC patients' symptom burden. It is possible that the relationship between perceived stress and self-report symptoms – which is frequently observed - is due to a

psychological negative reporting bias. That is, patients that report more severe symptoms in self-report assessments may be more likely to report greater threat, stress and negative mood (Macleod et al., 2002; Sato & Kawahara, 2011). If this is the case, then UC clinical care may benefit from assessing and monitoring patients' emotional and psychological well-being alongside the standard primary provision of care. Indeed, both doctors and patients believe they would benefit if gastroenterology teams included a psychologist (Jimenez et al., 2017).

A novelty of the current study is the use of the IBD disk which allows symptoms from bodily, social and psychological domains of UC to be examined. For negative affect and perceived change in HR, a consistent association was observed for nearly all IBD disk items, suggesting these two psychological responses to stress are closely associated with general symptom burden in UC. Examining the individual items, body image was consistently associated with psychological responses including perceived threat, cognitive and somatic anxiety intensity, negative affect, change in HR, and perceived stressfulness during the stress task. It is not clear why these associations with a psychological domain of symptom burden and a lack of association with bodily functions are apparent. One explanation is due to the socially evaluative nature of the stress task, where participants were recorded via video tape. Future work should continue to measure overall symptom burden, but also explore the physiological, psychological and social domains of UC burden which are essential to fully understand how stress impacts patients with UC.

Although the current study provides a detailed overview of cardiovascular, autonomic, inflammatory, and psychological stress mechanisms in UC patients, it is not without limitations. First, a relatively small sample size was used; however the sample is equal to the two previous studies investigating stress in IBD (Kuroki et al., 2011; Mawdsley et al., 2006),

and includes only UC, rather than a mixed UC/CD sample which reduces power (Camara et al., 2009; Keefer et al., 2008). Second, only a small sub-sample of patients provided a stool sample, and no control comparison was used for FCAL analyses to demonstrate whether stress increases FCAL in healthy individuals, or whether similar variability in FCAL would be observed across two control, non-laboratory stress day. Thus, the FCAL data must be used as preliminary evidence. Third, many correlations were completed to examine the associations between UC symptom burden and stress related responses which increases the chance of Type 1 errors.

In conclusion, acute psychological stress appears to increase systemic and bowel specific inflammation and can induce negative psychological states in UC patients. UC patients experienced blunted vagal activity at rest and during stress which could increase symptoms and symptom burden for patients with UC. However, given the lack of associations between physiological markers during stress and UC burden, this mechanism is not supported. The current study revealed associations between acute stress-induced negative psychological states (negative affect, perceived threat, and perceived change in HR) and greater overall UC symptom burden. The findings suggest that psychological factors may play a more prominent role than physiological stress variables in the UC stress-symptom relationship. Given the beneficial effects of stress re-appraisal techniques on subjective self-reported stress in healthy populations (Liu, Ein, Gervasio, & Vickers, 2019), it is of clinical interest to examine whether psychological interventions aiming to alter psychological stress appraisals would alter self-reported UC disease symptoms and resulting symptom burden.

**Supplementary Table 5.1.** Correlation between IBD symptom burden and task physiological responses to acute psychological stress.

| | IBD DISK SUM | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cardiovascular responses | | | | | | | | | | | |
| Heart rate reactivity | .02 | .14 | -.10 | .24 | -.08 | -.14 | -.14 | .02 | -.15 | .13 | -.08 |
| Heart rate variability reactivity | -.16 | -.26 | -.08 | -.33 | .07 | .04 | -.06 | -.08 | .08 | -.27 | .08 |
| Pre-ejection period reactivity | -.27 | .04 | .16 | -.25 | .03 | -.40 | -.19 | -.12 | -.32 | -.05 | -.18 |
| Systolic blood pressure reactivity | .09 | -.26 | -.10 | -.01 | -.34 | .12 | .17 | .07 | .10 | .24 | .38 |
| Diastolic blood pressure reactivity | -.04 | -.16 | -.01 | .04 | -.34 | -.07 | -.05 | -.02 | -.24 | .27 | .19 |
| Inflammatory Responses | | | | | | | | | | | |
| IL-6 Reactivity (+30mins) | .18 | .10 | .47 | .39 | .16 | .16 | .01 | .10 | -.12 | -.08 | -.06 |
| IL-6 Reactivity (+60mins) | .19 | -.11 | .41 | .38 | -.01 | .41 | .19 | .18 | .12 | -.23 | -.17 |
| Faecal calprotectin reactivity | -.25 | -.05 | -.30 | -.32 | -.20 | .17 | -.06 | -.43 | -.18 | -.46 | .37 |

*Note:* 1. Abdominal pain 2. Regulating defecation 3. Interpersonal interactions 4. Education and work 5. Sleep 6. Energy 7. Emotions 8. Body image 9. Sexual functions 10. Joint pain

# Chapter 6

GENERAL DISCUSSION

The principal aim of this thesis was to examine factors associated with the individual differences in physiological and psychological responses to stress, and whether these stress responses can be altered through an acute exercise intervention. Secondly, this thesis explored whether stress contributes towards disease symptoms in patients with ulcerative colitis (UC).To examine this research question four empirical laboratory-based studies were completed using a range of interdisciplinary methodologies, including cardiovascular assessments, magnetic resonance imaging (MRI), psychological assessments, metabolic assessments and assessments of inflammatory markers.

## Summary of Results

**Brain morphology and cardiovascular reactivity.** The specific aim of Chapter 2 was to explore whether amygdala and hippocampus brain morphology associated with stressor-evoked cardiovascular reactivity. Using detailed cardiovascular assessments to a standardised psychological stress task and MRI, associations between brain morphology and stressor-evoked cardiovascular reactivity were revealed. The results suggest that the amygdala and hippocampus brain regions, which are centrally involved in threat perception, emotional regulation, and autonomic control are implicated as influencing the individual differences in cardiovascular responses to acute psychological stress.

**Acute exercise prior to acute psychological stress.** Chapter 2 outlined dispositional factors influencing cardiovascular reactivity, therefore it is of interest to examine whether it is possible to alter the cardiovascular responses to acute psychological stress. Consequently, Chapter 3 explored whether completing an acute bout of high intensity exercise prior to exposure to acute psychological stress influenced the cardiovascular responses to stress, as well as investigating how exercise influences the psychological responses to stress. Chapter

3's results demonstrated exercise reduced blood pressure, which was in line with similar reductions in somatic anxiety, yet beneficial effects were not demonstrated for cognitive anxiety or other psychological responses. In fact, exercise induced more negative primary and secondary appraisals and more negative mood in response to stress. These results suggest that cardiovascular and psychological responses may not be related, given the opposing effects observed. Given these findings it would be of interest to explore factors contributing towards the experience of negative psychological states during stress.

**Heart rate, perceived heart rate and anxiety.** Chapter 4 subsequently investigated how physiological changes during stress and perceptions of these physiological changes contribute towards the experience of cognitive and somatic anxiety intensity and perceived interpretation of anxiety symptoms. Across two studies, it was revealed that an individual's perception of their HR during stress is more closely associated with anxiety symptoms compared to actual HR reactivity. This highlights healthy individuals' perceptions of stress may be more important than the actual physiological stress response for influencing well-being. It would be interesting to explore whether psychological or physiological stress responses have a more important role influencing disease burden in patient populations where stress is posited to influence disease status and well-being.

**Stress and ulcerative colitis.** Chapter 5 therefore investigated the role of stress in patients with UC by exploring whether patients differ in psychological and physiological stress responses, and how these responses associate with UC symptom burden. Differences between groups for both physiological (inflammation, vagal activity) and psychological (perceived control) stress responses were observed. However, no associations were evident between physiological stress responses and UC disease burden. Significant positive associations were found between total UC symptom burden and psychological stress

responses. This Chapter in a clinical population adds to the previous chapters in healthy populations, demonstrating apparent contrasts between physiological and psychological stress responses and how they may relate to health. The results highlight the importance of assessing psychological state when trying to understand the relationship between stress and health.

## Implications

Using four empirical chapters each with different experimental designs and participant populations, several important overarching implications emerge beyond those addressed in each specific empirical chapter.

**Dissociation between physiological and psychological responses.** One of the most consistent themes emerging throughout the thesis was the lack of coherence across physiological and psychological responses to acute psychological stress. For example, Chapter 3 revealed that following acute exercise there were attenuations in systolic blood pressure responses, yet greater primary appraisals of stress including perceived threat, stressfulness, and difficulty were reported during stress following exercise. Thus, the cardiovascular and psychological responses following exercise have opposing effects; beneficial effects for blood pressure but detrimental effects for appraisals and mood. Chapter 4 then demonstrated that perceptions of HR change, rather than actual HR change was more closely related to measures of both cognitive and somatic anxiety during acute psychological stress, again suggesting a lack of association across physiological and psychological measures. Further, in Chapter 5, patients with UC demonstrated that physiological responses during acute psychological stress including cardiovascular activity and markers of inflammation were unrelated to disease burden, yet psychological stress responses were

associated with greater disease burden, emphasising the importance of psychological factors in disease exacerbation.

Although it is inviting to suggest that physiological and psychological response systems are tightly linked, the current thesis adds to the literature suggesting there is a lack of coherence across systems (Ali, Nitschke, Cooperman, & Pruessner, 2017; Campbell & Ehlert, 2012; Feldman et al., 1999; Grossman et al., 2001; Hilmert & Kvasnicka, 2010). Importantly, throughout this thesis the lack of coherence across systems is demonstrated across different methodological designs and measures. This raises interesting questions regarding the relationship between physiological and psychological responses to stress and their respective importance towards health. It is well reported that physiological responses to stress are related to poorer future health outcomes (Chida & Steptoe, 2010; Steptoe & Kivimaki, 2012), however in the current thesis by using novel and detailed assessments it is highlighted that perceptions of stress and the psychological responses may be equally important towards health and well-being.

The outlined dissociations in psychological and physiological stress responses throughout this thesis have important implications for the development of interventions for health. That is, health is not just physiological or just psychological, but rather health is a complex interplay between physical and mental well-being. Interventions must account for the potentially opposing effects that treatment may induce on individuals as demonstrated in Chapter 3. For example, high intensity exercise was used in Chapter 3, founded in work demonstrating the greater the intensity exercise, the greater the attenuation in blood pressure responses (Hamer et al., 2006), yet this intensity induced negative effects on psychological states. Consequently Chapter 3 demonstrates the importance of assessing both psychological as well as physiological outcomes when investigating stress reactivity.

Together results from Chapter 3, Chapter 4 and Chapter 5 highlight that researchers must be mindful that physiological and psychological states may associate with health and well-being in different patterns. As research continues to investigate the relationship between stress and health, an interdisciplinary approach must be used with study outcomes accounting for the overall impact on both the mind and body.

**The importance of the brain during acute psychological stress.** It could be that trait biological factors (i.e., brain morphology) may be more important for influencing cardiovascular responses during stress rather than state psychological responses. Indeed, Chapter 2 demonstrates that an individual's brain morphology, specifically the amygdala and hippocampus brain regions could be an important factor underlying individual differences in cardiovascular reactivity. The notion that the mind is embodied is a long-standing concept and several models of psychological stress have emphasised that the brain is an integral regulator of stress reactivity (Gianaros & Jennings, 2018; Gianaros & Wager, 2015; Ginty et al., 2017; McEwen & Gianaros, 2011). In particular, Lovallo (2005) proposed a three level model of cardiovascular reactivity; Level I suggested corticolimbic structures could influence stress reactivity; Level II suggested the brainstem and hypothalamus organises descending projections influencing stress reactivity; Level III suggested peripheral factors influence the outcome of the above descending information and consequently alter reactivity (Lovallo, 2005).

Chapter 2 supports, in part, Lovallo's model of cardiovascular reactivity by demonstrating associations with brain structures in Level I of the model. In agreement with Lovallo (2005), Chapter 2 is the first study to examine how cardiovascular measures including HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), stroke volume (SV), cardiac output (CO) and total peripheral resistance

(TPR) during acute psychological stress are associated with brain morphology. Only one previous study has explored the association between brain morphology and stressor-evoked cardiovascular reactivity, but MAP was the only cardiovascular measurement (Gianaros et al., 2008). Thus, the current empirical work provides a more detailed assessment of the relationship the brain structure and cardiovascular reactivity during stress. Integrating MRI and cardiovascular assessments is novel, particularly using Doppler echocardiography, in a literature predominantly reliant on ECG and blood pressure assessments. It would be interesting to explore whether these corticolimbic structures involved in cognitive appraisal, processing, and ascribing actions, are related to other important outcome measures including psychological and inflammatory markers during acute psychological stress. If these brain structures, or other corticolimbic regions relate to a myriad a stress markers (i.e., cardiovascular reactivity, inflammation, HPA activity), a biological susceptibility model for stress could be developed. From a health perspective, identifying biological characteristics that may predispose individuals to the expression of heightened stress has a significant potential impact to identify individuals who have a greater susceptibility to experience stress phenotypes.

Interestingly, the hypothesis by Lovallo (2005) that stress reactivity is influenced by three levels may shed light on the weak relationship between appraisals/psychological responses and physiological stress reactivity. That is, while stress appraisals are generated in corticolimbic brain regions (Level I; Lovallo, 2005), physiological responses as identified in Chapter 2 are also influenced by corticolimbic structures. Thus, while psychological and physiological responses may both originate in the Level I brain structures, the lack of coherence between these outcomes might be explained by the fact cardiovascular reactivity during stress is further influenced by substantial downstream interference, i.e., level II (the

brainstem) and level III (the periphery) (Lovallo, 2005). Based on this observation, extension of the results reported in Chapter 2 would benefit by integrating structural MRI and cardiovascular reactivity with additional measures. In particular, measures of endothelial function with flow-mediated dilation (FMD) would be an insightful addition to assess level III factors in the periphery which could relate to health. Indeed, the FMD response to stress is associated with adverse cardiovascular events (Poitras & Pyke, 2013; Pyke & Tschakovsky, 2005), and if integrated with structural MRI and cardiovascular reactivity, it would be interesting to test the three level model proposed by Lovallo (2005), exploring whether clusters of individuals with altered brain morphology, cardiovascular and endothelial responses during stress are apparent.

**Disease burden; the psychological impact on a physiological disease.** A growing body of cross-sectional, and prospective research with large patient samples has added to the evidence of an association between life stress and inflammatory bowel disease (IBD) symptom activity (Bernstein, 2017; Camara et al., 2009; Sajadinejad et al., 2012; Sexton et al., 2017; Targownik et al., 2015; Triantafillidis et al., 2013; Walker et al., 2008). What is particularly interesting is the apparent lack of relationship between objective markers of disease activity (i.e., intestinal inflammation) and perceptions of stress (Sexton et al., 2017; Targownik et al., 2015). Rather, consistent associations are demonstrated between self-reported disease activity questionnaires and self-reported perceptions of life stress (Bernstein et al., 2010; Camara et al., 2009). Interestingly Chapter 5 revealed no associations between any physiological stress marker and UC disease burden, while associations between psychological stress responses and UC disease burden were observed.

While clinical diagnosis utilises blood inflammatory markers, stool samples and biopsies from the colon, all physiological objective markers, the disease is also characterised

by significant psychological burden (Burisch, Jess, Martinato, & Lakatos, 2013; Hauser et al., 2011; Nahon et al., 2012; Sajadinejad et al., 2012). Chapter 5's results add to the literature demonstrating there is a close connection between health complaints, stress, and negative affectivity, but specifically identify this relationship is apparent in UC. Indeed, David Watson and Pennebaker (1989) demonstrated that negative affect is strongly related to health complaints, but not long-term health status, and a self-reporting bias may occur where greater symptoms are reported in the absence of changes in disease outcome (Macleod et al., 2002). Alternatively, psychological factors (e.g., stress, negative affect) may have indirect effects on disease activity, through alterations in behaviour change such as reduced medication adherence, loss of social interaction, reductions in physical activity among others (Ogden, 2012). Therefore, negative psychological states could be impacting disease burden through non-direct effects, but it is also possible that individuals reporting greater stress may simultaneously be reporting greater disease without altering symptoms.

Regardless of actual disease activity, Chapter 5 adds to the above phenomenon highlighting that negative psychological responses to stress in UC is related to reporting greater disease burden, with no associations evident with physiological responses. Given that UC is a patient population with heightened reported stress (Sajadinejad et al., 2012; Triantafillidis et al., 2013; Walker et al., 2008), it raises the question of whether there is a need for clinical care to incorporate psychological management as part of holistic medical practice. That is, clinical care of UC relies on objective markers to ascertain disease state and guide treatment, but if psychological factors have an important contributory role in exacerbating UC, regular monitoring of current psychological states (e.g., anxiety, life stress and mood) could identify patients who might be at risk of relapsing. As a result, support through current treatment pathways, or through psychological support and interventions,

including stress reduction techniques may reduce the risk of relapse as well as the burden on the health care system if identified early. For example, in a meta-analysis assessing the efficacy of stress reappraisal interventions in healthy individuals, Liu et al. (2019) reported that overall subjective responsivity to stress is effectively attenuated, yet beneficial effects are not observed for physiological stress responses. Examining whether patients with UC report lower disease symptoms and burden following a stress reappraisal intervention would be insightful. If patients with UC report attenuated subjective responses to stress, and lower disease symptoms, this would emphasise a psychological mechanism relating stress and UC symptom exacerbation.

**Strengths and Limitations**

Strengths and limitations for each study have been discussed in the Discussion section for each empirical chapter and consequently this section relates to common strengths and limitations across the chapters of this thesis. First, one of the major strengths of this thesis is the use of interdisciplinary methodologies. Across the four laboratory studies electrocardiography, impedance cardiography, Doppler echocardiography, blood pressure monitoring, structural MRI, trait and state psychological assessment, assessment of inflammatory markers and metabolic assessments were utilised to examine the physiological and psychological responses to acute psychological stress and complex interplay between such responses. This integration of cardiovascular, neural, inflammatory and psychological assessments provides a comprehensive investigation and holistic understanding of how individuals respond to stress. There is a need to understand how simultaneous systems inter-relate, with several authors stating that this should be fundamental within psychophysiology research (Andrews, Ali, & Pruessner, 2013; Rohleder, 2019). By using these combinations of measures, this thesis explores novel relationships across the different stress axis.

The use of validated and reliable psychological stress tasks across all chapters is another strength of this thesis. This is evidenced by significant perturbations in cardiovascular and inflammatory activity as well as reporting of stress and negative psychological states, suggesting that all stress tasks used were effective at inducing stress. While Chapters 2, 3 and 5 each used a single stress task, Chapter 4 used an aggregation method proposed by (Kamarck et al., 2000; Kamarck et al., 1993) where multiple stress tasks are employed with differing psychological demands and scores are aggregated to create a single stress task composite score. Together these single and aggregated stress task methodologies provide a rigorous, reliable and valid approach to assess acute psychological responses.

The current thesis is not without limitations. First, all studies were laboratory-based research. While this provided a definitely controlled study design using a standardised acute psychological stress paradigm (Chapters 2-5) and the ability to counterbalance designs (Chapter 3) for effects to be observed, the laboratory-based setting may reduce the generalisability to real life settings. However, the use of social evaluation within stress paradigms, which is utilised across all chapters in this thesis, is argued to improve the ecological validity of laboratory stress (Linden, Rutledge, & Con, 1998).

Second, a limitation of the current thesis is the primarily cross-sectional nature of the research and inherent inability to prove causation (with the exception of Chapter 3). Given the relatively novel research questions being investigated, it is necessary to first examine proof of principle before designing more complex longitudinal research. This thesis will be able to directly inform longitudinal research which will ultimately inform health practice, such as investigating how brain morphology and stress reactivity relate, as well the relationship between stress reactivity and disease activity in inflammatory bowel disease over time.

## Future Directions

The broader future directions not previously mentioned in each chapter will now be discussed. As outlined, stress can lead to a range of adverse health outcomes and is ever increasing in modern society making it a global health challenge. Understanding pathways to experiencing heightened stress reactivity is therefore of great importance. Given the associations observed in Chapter 2 between reduced amygdala and hippocampus volume and exaggerated cardiovascular stress reactivity, future work should investigate whether the assessment of brain morphology has predictive capabilities for future stress reactivity or resilience. Specifically, assessing brain morphology during periods of development (i.e., adolescence) or prior to starting an important transition in life (e.g., leaving full-time education), followed by multiple follow-ups in the subsequent years would provide evidence for whether brain morphology can predict stress related phenotypes and could be used as a biological identification tool for at-risk individuals. For example, studies are using similar methodologies in paediatric populations with brain injuries, using structural MRI and machine learning to predict whether children will develop early life developmental difficulties (King, Ellis, Seri, & Wood, 2019; Mateos-Pérez et al., 2018). Translating this novel neuroimaging and longitudinal research design into the stress literature would be invaluable, particularly for individuals beginning careers entailing high stress such as the military.

The results throughout this thesis demonstrate multiple systems are activated during stress involving feedback and feed-forward loops controlling response outputs. As demonstrated in Chapter 3, 4, and 5 there is little apparent coherence across these response and highlights the complexity of investigating multiple systems in response to stress. Consequently emphasis is needed for future studies to measure multiple bodily systems simultaneously (i.e., cardiovascular, HPA, neural, immune, psychological). One approach that

will provide an invaluable understanding of individual differences in stress reactivity is the use of technology to assess both psychological and physiological stress measures. Specifically, the use of mobile phone technology has seen the rise in ecological momentary assessments to measure real-time daily mood states through self-report questionnaires (Yang, Ryu, Han, Oh, & Choi, 2018). Further, cutting edge development of tools which record the language used on mobile phones via the phone's keyboard, time spent socialising, and geo-tagging which records time away from home, provides naturalistic behavioural data (Byrne et al., 2019). This development of mobile app-based behavioural information, can provide real-world data on psychological states (Byrne et al., 2019).

When this technology is coupled with physiological measures which can be easily obtained by participants, detailed analyses can be completed to explore how daily stress markers interact. For example, participants can provide daily saliva samples which can be analysed for HPA activity (e.g., cortisol) as well as sympathetic nervous system activity (e.g., salivary alpha amylase). Furthermore, through the use of wearable technology such as smart watches, continuous data on cardiovascular activity (e.g., HR) as well as physical activity levels can be measured (Piwek, Ellis, Andrews, & Joinson, 2016). Integrating these developments of technology into psychological science is an exciting avenue which will help examine the day-to-day associations across multiple systems in response to real-life stress.

Applying the above methods to UC specifically, stimulates interesting future research following the results from Chapter 5. It is evident that individuals with UC do not experience augmented stress responses, yet in UC, patients' perceived stress is related to a greater disease burden. However, these findings are only of a cross-sectional nature. What would be particularly interesting would be to investigate the dynamics between stress and disease symptoms on a day-to-day basis. The use of ambulatory physiological methods in

combination with daily ecological momentary assessments allows detailed measurements of real time experiences, limiting the effects of recall bias (Shiffman, Stone, & Hufford, 2008). Using advanced multi-level modelling techniques would allow a detailed examination of how stress and disease activity relate to each other throughout a single day, as well as over multiple days on a within-individual basis. This methodology would prove insightful into understanding how stress impacts health in UC.

Regardless of whether studies assessing the role of stress reactivity explore this in the laboratory or in a naturalistic setting, it is imperative for future research where possible, to implement interdisciplinary methods. It is currently unclear how acute stress turns into chronic stress (Rohleder, 2019), but by integrating novel methodologies including measures of brain function, autonomic activity, immunological activity, neuroendocrine activity and detailed psychological reporting, assessed using cross-sectional as well as longitudinal methodologies, this will provide a more comprehensive understanding of the relationship between stress and health**.**

An interesting hypothesis in the stress-reactivity literature is the notion that the pattern of adaptation to stress has important implications for health. Specifically, Hughes, Lu, and Howard (2018) proposed that there are four profiles of cardiovascular adaptation to stress. These represent, persistent reactor, persistent blunter, habituator and sensitizer, which have potential separate predictive outcomes for health. A persistent reactor exhibits a substantial response to repeated stress exposures, and a repeated blunter in contrast exhibits repeated low responses. A habituator is proposed to be the most optimal stress phenotype, demonstrating an initial response to stress, but upon repeated exposures a much lower response is expressed. Fourth, a sensitizer is proposed to lead to the least optimal health outcomes, characterised by an initial stress response, which is amplified upon repeated exposures (Hughes et al., 2018).

This notion of stress adaptation has important applications throughout this thesis. For example, extending Chapter 3, it would be interesting to examine whether individuals who are categorised into each of the four adaptation profiles, would experience similar alterations in stress reactivity following an acute bout of exercise. Similarly, using Chapter 4 as a foundation, it would interesting to see after repeated stress exposures whether individuals have temporal stability in how they perceive their HR to change, and whether a relationship between perceived HR and anxiety is specific to each of the four profiles of adaptation.

Furthermore, it would be particularly interesting to explore in IBD whether patients are characterised by a certain response profile. This analysis brings advantages to the predominant single stress exposure method, as patterns of reactivity over time can be assessed which could provide a more detailed understanding of how repeated acute stress can influence individuals. Patients with IBD are exposed to repeated acute stressors throughout the day such as struggling to leave the house due to the stress of being near known toilet facilities or suffering from faecal incontinence are reasons why greater stress is reported compared to healthy individuals (Mikocka-Walus et al., 2016; Sajadinejad et al., 2012). If patients with IBD are characterised by a 'sensitizer' or 'persistent' profile, coupled with the repeated exposure to stress resulting from the disease, this would highlight a potential mechanism explaining why stress has significant implications on disease activity in IBD.

Finally, there are some important implications arising from Chapter 3 which should inform interventions. High intensity exercise was selected based on evidence implicating the greater the intensity of exercise, the greater the attenuation in blood pressure reactivity to stress. However, whilst blood pressure reactivity was reduced, the exercise induced primarily negative effects on psychological states. There is literature demonstrating that rather than imposing a pre-set exercise intensity upon participants, exercise performed at self-selected

workloads yields the greatest benefit for psychological states (Ekkekakis, 2009; Szabo, 2003). It would be interesting to explore whether an exercise intervention allowing participants to choose their preferred exercise intensity would see benefits in both physiological and psychological responses to stress to have the greatest overall impact on health.

## Conclusions

In conclusion, this thesis encompassing four empirical chapters using laboratory based studies, examined the individual differences in physiological and psychological responses to acute psychological stress in healthy and patient populations. It was demonstrated that brain morphology is associated with cardiovascular stress reactivity, and while acute high intensity exercise may reduce blood pressure responses to stress, the high intensity nature of this exercise has detrimental effects on psychological state during stress. Further, perceived change in physiological responses during stress was more strongly associated with anxiety experienced during stress than actual physiological changes. Finally, in patients with UC, psychological factors during stress were related to disease burden, yet a lack of associations between physiological responses and disease burden were apparent. This thesis provides further evidence for the complex individual differences in physiological and psychological responses during acute psychological stress, and emphasises the need for research to include measures of both physiological parameters as well as psychological parameters to appropriately examine the role of stress in influencing health.

## List of References


Aghajani, M., Veer, I. M., van Hoof, M. J., Rombouts, S., van der Wee, N. J., & Vermeiren, R. (2016). Abnormal functional architecture of amygdala-centered networks in adolescent posttraumatic stress disorder. *Human Brain Mapping, 37*(3), 1120-1135. doi:10.1002/hbm.23093

AlAbsi, M., Bongard, S., Buchanan, T., Pincomb, G. A., Licinio, J., & Lovallo, W. R. (1997). Cardiovascular and neuroendocrine adjustment to public speaking and mental arithmetic stressors. *Psychophysiology, 34*(3), 266-275. doi:10.1111/j.1469-8986.1997.tb02397.x

Alderman, B. L., Arent, S. M., Landers, D. M., & Rogers, T. J. (2007). Aerobic exercise intensity and time of stressor administration influence cardiovascular responses to psychological stress. *Psychophysiology, 44*(5), 759-766.

Ali, N., Nitschke, J. P., Cooperman, C., & Pruessner, J. C. (2017). Suppressing the endocrine and autonomic stress systems does not impact the emotional stress experience after psychosocial stress. *Psychoneuroendocrinology, 78*, 125-130. doi:10.1016/j.psyneuen.2017.01.015

Allen, M. T. (2008). Laboratory Tasks for Cardiovascular Reactivity Research. Retrieved from http://www.macses.ucsf.edu/Research/Psychosocial/reactivitytable.php

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders: DSM-IV-TR* (4th ed.). Washington.

Anderson, E. R., & Hope, D. A. (2009). The relationship among social phobia, objective and perceived physiological reactivity, and anxiety sensitivity in an adolescent population. *Journal of Anxiety Disorders, 23*(1), 18-26. doi:10.1016/j.janxdis.2008.03.011

Andrews, J., Ali, N., & Pruessner, J. C. (2013). Reflections on the interaction of psychogenic stress systems in humans: The stress coherence/compensation model. *Psychoneuroendocrinology, 38*(7), 947-961. doi:https://doi.org/10.1016/j.psyneuen.2013.02.010

Antoni, M. H., Lutgendorf, S. K., Cole, S. W., Dhabhar, F. S., Sephton, S. E., McDonald, P. G., . . . Sood, A. K. (2006). The influence of bio-behavioural factors on tumour biology: pathways and mechanisms. *Nat Rev Cancer, 6*(3), 240-248. doi:10.1038/nrc1820

Armstrong, K. A., & Khawaja, N. G. (2002). Gender differences in anxiety: An investigation of the symptoms, cognitions, and sensitivity towards anxiety in a nonclinical population. *Behavioural and Cognitive Psychotherapy, 30*(2), 227-231. doi:10.1017/s1352465802002114

Balanos, G. M., Phillips, A. C., Frenneaux, M. P., McIntyre, D., Lykidis, C., Griffin, H. S., & Carroll, D. (2010). Metabolically exaggerated cardiac reactions to acute psychological stress: The effects of resting blood pressure status and possible underlying mechanisms. *Biological Psychology, 85*(1), 104-111. doi:10.1016/j.biopsycho.2010.06.001

Bandelow, B., & Michaelis, S. (2015). Epidemiology of anxiety disorders in the 21st century. *Dialogues in clinic neuroscience, 17*(3), 327-335. doi:0.1016/j.mppsy.2007.01.007.

Barnett, P. A., Spence, J. D., Manuck, S. B., & Jennings, J. R. (1997). Psychological stress and the progression of carotid artery disease. *Journal of Hypertension, 15*(1), 49-55. doi:http://dx.doi.org/10.1097/00004872-199715010-00004

Bartholomew, J. B., Morrison, D., & Ciccolo, J. T. (2005). Effects of Acute Exercise on Mood and Well-Being in Patients with Major Depressive Disorder. *Medicine &*

*Science in Sports & Exercise, 37*(12), 2032-2037. doi:10.1249/01.mss.0000178101.78322.dd

Basso, J. C., & Suzuki, W. A. (2017). The Effects of Acute Exercise on Mood, Cognition, Neurophysiology, and Neurochemical Pathways: A Review. *Brain Plasticity, 2*, 127-152.

Baum, A., & Posluszny, D. M. (1999). Health psychology: mapping biobehavioral contributions to health and illness. *Annu Rev Psychol, 50*, 137-163. doi:10.1146/annurev.psych.50.1.137

Beauchet, O., Celle, S., Roche, F., Bartha, R., Montero-Odasso, M., Allali, G., & Annweiler, C. (2013). Blood pressure levels and brain volume reduction: a systematic review and meta-analysis. *Journal of Hypertension, 31*(8), 1502-1516. doi:http://dx.doi.org/10.1097/HJH.0b013e32836184b5

Beh, H. C. (1998). Cardiovascular reactivity to psychological stressors. *Australian Journal of Psychology, 50*(1), 49-54. doi:10.1080/00049539808257531

Beissner, F., Meissner, K., Bar, K. J., & Napadow, V. (2013). The autonomic brain: an activation likelihood estimation meta-analysis for central processing of autonomic function. *Journal of Neuroscience, 33*(25), 10503-10511. doi:http://dx.doi.org/10.1523/JNEUROSCI.1103-13.2013

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society, 57*(1), 289-300.

Benschop, R. J., & Schedlowski, M. (1999). Acute Psychological Stress. In M. Schedlowski & U. Tewes (Eds.), *Psychoneuroimmunology: An Interdisciplinary Introduction* (pp. 293-306). Boston, MA: Springer US.

Benvenutti, M. J., Alves, E. D. S., Michael, S., Ding, D., Stamatakis, E., & Edwards, K. M. (2017). A single session of hatha yoga improves stress reactivity and recovery after an acute psychological stress task-A counterbalanced, randomized-crossover trial in healthy individuals. *Complement Ther Med, 35*, 120-126. doi:10.1016/j.ctim.2017.10.009

Bernstein, C. N. (2016). Psychological Stress and Depression: Risk Factors for IBD? *Dig Dis, 34*(1-2), 58-63. doi:10.1159/000442929

Bernstein, C. N. (2017). The Brain-Gut Axis and Stress in Inflammatory Bowel Disease. *Gastroenterol Clin North Am, 46*(4), 839-846. doi:10.1016/j.gtc.2017.08.006

Bernstein, C. N., Singh, S., Graff, L. A., Walker, J. R., Miller, N., & Cheang, M. (2010). A Prospective Population-Based Study of Triggers of Symptomatic Flares in IBD. *American Journal of Gastroenterology, 105*(9), 1994-2002. doi:10.1038/ajg.2010.140

Bernstein, C. N., Walker, J. R., & Graff, L. A. (2006). On studying the connection between stress and IBD. *American Journal of Gastroenterology, 101*(4), 782-785. doi:10.1111/j.1572-0241.2006.00474.x

Bibbey, A. (2015). *Constitutional and behavioural correlates of individual differences in biological stress reactivity.* (Doctor of Philosophy), The University of Birmingham,

Bibbey, A., Carroll, D., Roseboom, T. J., Phillips, A. C., & de Rooij, S. R. (2013). Personality and physiological reactions to acute psychological stress. *Int J Psychophysiol, 90*(1), 28-36. doi:10.1016/j.ijpsycho.2012.10.018

Bitton, A., Dobkin, P. L., Edwardes, M. D., Sewitch, M. J., Meddings, J. B., Rawal, S., . . . Wild, G. E. (2008). Predicting relapse in Crohn's disease: a biopsychosocial model. *Gut, 57*(10), 1386-1392. doi:10.1136/gut.2007.134817

Bitton, A., Sewitch, M. J., Peppercorn, M. A., Edwardes, M. D. D., & Shah, S. (2003). Psychosocial determinants of relapse in ulcerative colitis: A longitudinal study. *American Journal of Gastroenterology, 98*(10), 2203-2208. doi:10.1016/s0002-9270(03)00750-0

Bjelland, I., Dahl, A. A., Haug, T. T., & Neckelmann, D. (2002). The validity of the Hospital Anxiety and Depression Scale. An updated literature review. *J Psychosom Res, 52*(2), 69-77.

Bonaz, B., Bazin, T., & Pellissier, S. (2018). The Vagus Nerve at the Interface of the Microbiota-Gut-Brain Axis. *Front Neurosci, 12*, 49. doi:10.3389/fnins.2018.00049

Bonaz, B., & Bernstein, C. N. (2013). Brain-Gut Interactions in Inflammatory Bowel Disease. *Gastroenterology, 144*(1), 36-49. doi:10.1053/j.gastro.2012.10.003

Bosch, J. A., de Geus, E. J. C., Carroll, D., Goedhart, A. D., Anane, L. A., Veldhuijzen van Zanten, J. J. C. S., . . . Edwards, K. M. (2009). A General Enhancement of Autonomic and Cortisol Responses During Social Evaluative Threat. *Psychosomatic Medicine, 71*(8), 877-885. doi:10.1097/PSY.0b013e3181baef05

Boyle, M. P., Brewer, J. A., Funatsu, M., Wozniak, D. F., Tsien, J. Z., Izumi, Y., & Muglia, L. J. (2005). Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. *Proceedings of the National Academy of Sciences of the United States of America, 102*(2), 473-478. doi:http://dx.doi.org/10.1073/pnas.0406458102

Brindle, R. C., Ginty, A. T., Phillips, A. C., & Carroll, D. (2014). A tale of two mechanisms: A meta-analytic approach toward understanding the autonomic basis of cardiovascular reactivity to acute psychological stress. *Psychophysiology, 51*(10), 964-976. doi:10.1111/psyp.12248

Broman-Fulks, J. J., Kelso, K., & Zawilinski, L. (2015). Effects of a Single Bout of Aerobic Exercise Versus Resistance Training on Cognitive Vulnerabilities for Anxiety Disorders. *Cognitive Behaviour Therapy, 44*(4), 240-251. doi:10.1080/16506073.2015.1020448

Brotman, D. J., Golden, S. H., & Wittstein, I. S. (2007). The cardiovascular toll of stress. *Lancet, 370*(9592), 1089-1100. doi:10.1016/s0140-6736(07)61305-1

Brownley, K. A., Hinderliter, A. L., West, S. G., Girdler, S. S., Sherwood, A., & Light, K. C. (2003). Sympathoadrenergic mechanisms in reduced hemodynamic stress responses after exercise. *Medicine and science in sports and exercise, 35*(6), 978-986. doi:10.1249/01.mss.0000069335.12756.1b

Brzozowski, B., Mazur-Bialy, A., Pajdo, R., Kwiecien, S., Bilski, J., Zwolinska-Wcislo, M., . . . Brzozowski, T. (2016). Mechanisms by which Stress Affects the Experimental and Clinical Inflammatory Bowel Disease (IBD): Role of Brain-Gut Axis. *Current Neuropharmacology, 14*(8), 892-900. doi:10.2174/1570159x14666160404124127

Buckner, R. L., Head, D., Parker, J., Fotenos, A. F., Marcus, D., Morris, J. C., & Snyder, A. Z. (2004). A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. *NeuroImage, 23*(2), 724-738. doi:http://dx.doi.org/10.1016/j.neuroimage.2004.06.018

Burisch, J., Jess, T., Martinato, M., & Lakatos, P. L. (2013). The burden of inflammatory bowel disease in Europe. *Journal of Crohn's and Colitis, 7*(4), 322-337. doi:https://doi.org/10.1016/j.crohns.2013.01.010

Butterworth, P., Cherbuin, N., Sachdev, P., & Anstey, K. J. (2012). The association between financial hardship and amygdala and hippocampal volumes: results from the PATH

through life project. *Social Cognitive and Affective Neuroscience, 7*(5), 548-556. doi:10.1093/scan/nsr027

Byrne, M. L., Horn, S. R., Barnes, M. L., Lind, M. N., Mills, K. L., Nelson, B. W., . . . Allen, N. B. (2019). Mobile measurement of psychological stress: an exploratory pilot study comparing passive mobile phone sensing data with biological and self-reported stress. *Psychosomatic Medicine, 81*(4), A163-A163.

Cacioppo, J. T., Berntson, G. G., Binkley, P. F., Quigley, K. S., Uchino, B. N., & Fieldstone, A. (1994). Autonomic cardiac control. II. Noninvasive indexes and basal response as revealed by autonomic blockades. *Psychophysiology, 31*(6), 586-598. doi:10.1111/j.1469-8986.1994.tb02351.x

Cahn, W., Pol, H. E. H., Lems, E. B., van Haren, N. E., Schnack, H. G., van der Linden, J. A., . . . Kahn, R. S. (2002). Brain volume changes in first-episode schizophrenia: a 1-year follow-up study. *Archives of General Psychiatry, 59*(11), 1002-1010. doi:http://dx.doi.org/10.1001/archpsyc.59.11.1002

Camara, R. J., Ziegler, R., Begre, S., Schoepfer, A. M., & von Kanel, R. (2009). The Role of Psychological Stress in Inflammatory Bowel Disease: Quality Assessment of Methods of 18 Prospective Studies and Suggestions for Future Research. *Digestion, 80*(2), 129-139. doi:10.1159/000226087

Campbell, J., & Ehlert, U. (2012). Acute psychosocial stress: Does the emotional stress response correspond with physiological responses? *Psychoneuroendocrinology, 37*(8), 1111-1134. doi:10.1016/j.psyneuen.2011.12.010

Carroll, D., Ginty, A. T., Der, G., Hunt, K., Benzeval, M., & Phillips, A. C. (2012). Increased blood pressure reactions to acute mental stress are associated with 16-year

cardiovascular disease mortality. *Psychophysiology, 49*(10), 1444-1448. doi:http://dx.doi.org/10.1111/j.1469-8986.2012.01463.x

Carroll, D., Ginty, A. T., Painter, R. C., Roseboom, T. J., Phillips, A. C., & de Rooij, S. R. (2012). Systolic blood pressure reactions to acute stress are associated with future hypertension status in the Dutch Famine Birth Cohort Study. *International Journal of Psychophysiology, 85*(2), 270-273. doi:http://dx.doi.org/10.1016/j.ijpsycho.2012.04.001

Carroll, D., Phillips, A. C., & Balanos, G. M. (2009). Metabolically exaggerated cardiac reactions to acute psychological stress revisited. *Psychophysiology, 46*(2), 270-275. doi:10.1111/j.1469-8986.2008.00762.x

Carroll, D., Phillips, A. C., Hunt, K., & Der, G. (2007). Symptoms of depression and cardiovascular reactions to acute psychological stress: Evidence from a population study. *Biological Psychology, 75*(1), 68-74. doi:10.1016/j.biopsycho.2006.12.002

Carroll, D., Ring, C., Hunt, K., Ford, G., & MacIntyre, S. (2003). Blood pressure reactions to stress and the prediction of future blood pressure: Effects of sex, age, and socioeconomic position. *Psychosomatic Medicine, 65*(6), 1058-1064. doi:10.1097/01.PSY.0000097330.58739.26

Carroll, D., Smith, G. D., Shipley, M. J., Steptoe, A., Brunner, E. J., & Marmot, M. G. (2001). Blood pressure reactions to acute psychological stress and future blood pressure status: A 10-year follow-up of men in the Whitehall II study. *Psychosomatic Medicine, 63*(5), 737-743.

Chamberlain, S. A., & Hale, B. D. (2007). Competitive state anxiety and self-confidence: Intensity and direction as relative predictors of performance on a golf putting task.

*Anxiety, Stress & Coping: An international Journal, 20*(2), 197-207. doi: 10.1080/10615800701288572

Charles, S. T., Piazza, J. R., Mogle, J., Sliwinski, M. J., & Almeida, D. M. (2013). The Wear and Tear of Daily Stressors on Mental Health. *Psychological Science, 24*(5), 733-741. doi:10.1177/0956797612462222

Chida, Y., & Steptoe, A. (2010). Greater Cardiovascular Responses to Laboratory Mental Stress Are Associated With Poor Subsequent Cardiovascular Risk Status A Meta-Analysis of Prospective Evidence. *Hypertension, 55*(4), 1026-U1368. doi:http://dx.doi.org/10.1161/HYPERTENSIONAHA.109.146621

Clark, D. M., Salkovskis, P. M., Ost, L. G., Breitholtz, E., Koehler, K. A., Westling, B. E., . . . Gelder, M. (1997). Misinterpretation of body sensations in panic disorder. *Journal of Consulting and Clinical Psychology, 65*(2), 203-213. doi:10.1037/0022-006x.65.2.203

Cohen, B. E., Edmondson, D., & Kronish, I. M. (2015). State of the Art Review: Depression, Stress, Anxiety, and Cardiovascular Disease. *Am J Hypertens, 28*(11), 1295-1302. doi:10.1093/ajh/hpv047

Cohen, R. A. (2007). Hypertension and cerebral blood flow - Implications for the development of vascular cognitive impairment in the elderly. *Stroke, 38*(6), 1715-1717. doi:http://dx.doi.org/10.1161/STROKEAHA.107.487165

Cohen, S., Hamrick, N., Rodriguez, M. S., Feldman, P. J., Rabin, B. S., & Manuck, S. B. (2000). The stability of and intercorrelations among cardiovascular, immune, endocrine, and psychological reactivity. *Annals of Behavioral Medicine, 22*(3), 171-179. doi:10.1007/bf02895111

Cohen, S., Janicki-Deverts, D., & Miller, G. E. (2007). Psychological Stress and Disease. *JAMA, 298*(14), 1685-1687. doi:10.1001/jama.298.14.1685

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24*(4), 385-396. doi:10.2307/2136404

Craske, M. G., Treanor, M., Conway, C. C., Zbozinek, T., & Vervilet, B. (2014). Maximising exposure therapy: An inhibitory learning approach. *Behavioral Research and Therapy, 58*, 10-23.

Crawford, J. R., & Henry, J. D. (2004). The positive and negative affect schedule (PANAS): construct validity, measurement properties and normative data in a large non-clinical sample. *Br J Clin Psychol, 43*(Pt 3), 245-265. doi:10.1348/0144665031752934

Critchley, H. D. (2005). Neural mechanisms of autonomic, affective, and cognitive integration. *Journal of Comparative Neurology, 493*(1), 154-166. doi:10.1002/cne.20749

Critchley, H. D. (2009). Psychophysiology of neural, cognitive and affective integration: fMRI and autonomic indicants. *International Journal of Psychophysiology, 73*(2), 88-94. doi:10.1016/j.ijpsycho.2009.01.012

Dai, W., Lopez, O. L., Carmichael, O. T., Becker, J. T., Kuller, L. H., & Gach, H. M. (2008). Abnormal regional cerebral blood flow in cognitively normal elderly subjects with hypertension. *Stroke, 39*(2), 349-354. doi:http://dx.doi.org/10.1161/STROKEAHA.107.495457

Dale, A. M., Fischl, B., & Sereno, M. I. (1999). Cortical Surface-Based Analysis I: Segmentation and Surface Reconstruction. *NeuroImage, 9*(2), 179-194. doi:http://dx.doi.org/10.1006/nimg.1998.0395

Damasio, A. R. (1996). The somatic marker hypothesis and the possible functions of the prefrontal cortex. *Philosophical Transactions of the Royal Society B-Biological Sciences, 351*(1346), 1413-1420. doi:10.1098/rstb.1996.0125

Dampney, R. A. L. (2015). Central mechanisms regulating coordinated cardiovascular and respiratory function during stress and arousal. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 309*(5), R429-R443. doi:10.1152/ajpregu.00051.2015

Darwin, C., Cummings, M., Duchenne, G., & John, M. (1872). *The expression of the emotions in man and animals*. London: John Murray.

de Geus, E. J. C., Willemsen, G. H. M., Klaver, C., & Vandoornen, L. J. P. (1995). Ambulatory Measurement of Respiratory Sinus Arrhythmia and Respiration Rate. *Biological Psychology, 41*(3), 205-227. doi:10.1016/0301-0511(95)05137-6

de Rooij, S. R. (2013). Blunted cardiovascular and cortisol reactivity to acute psychological stress: A summary of results from the Dutch Famine Birth Cohort Study. *International Journal of Psychophysiology, 91*(1), 21-27.

Degood, D. E., & Tait, R. C. (1987). The cognitive-somatic anxiety questionnaire - psychometric and validity data. *Journal of Psychopathology and Behavioral Assessment, 9*(1), 75-87. doi:10.1007/bf00961633

Dekker, J. M., Crow, R. S., Folsom, A. R., Hannan, P. J., Liao, D., Swenne, C. A., & Schouten, E. G. (2000). Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: the ARIC Study. Atherosclerosis Risk In Communities. *Circulation, 102*(11), 1239-1244. doi:10.1161/01.cir.102.11.1239

Dhabhar, F. S. (2009). Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *Neuroimmunomodulation, 16*(5), 300-317. doi:10.1159/000216188

Dogra, S., MacIntosh, L., O'Neill, C., D'Silva, C., Shearer, H., Smith, K., & Côté, P. (2018). The association of physical activity with depression and stress among post-secondary school students: A systematic review. *Mental Health and Physical Activity, 14*, 146-156. doi:https://doi.org/10.1016/j.mhpa.2017.11.001

Duda, J. L., Sedlock, D. A., Melby, C. L., & Thaman, C. (1988). The effects of physical activity level and acute exercise on heart rate and subjective response to a psychological stressor. *International Journal of Sports Pschology, 19*, 119-133.

Duffy, L. C., Zielezny, M. A., Marshall, J. R., Weiser, M. M., Phillips, J. F., Byers, T. E., . . . Ogra, P. L. (1991). Lag time between stress events and risk of recurrent episodes of inflammatory bowel disease. *Epidemiology (Cambridge, Mass.), 2*(2), 141-145. doi:10.1097/00001648-199103000-00009

Edelmann, R. J., & Baker, S. R. (2002). Self-reported and actual physiological responses in social phobia. *British Journal of Clinical Psychology, 41*, 1-14. doi:10.1348/014466502163732

Edmondson, D., Newman, J. D., Whang, W., & Davidson, K. W. (2013). Emotional triggers in myocardial infarction: do they matter? *European Heart Journal, 34*(4), 300-306.

Edwards, M. K., Rhodes, R. E., & Loprinzi, P. D. (2017). A Randomized Control Intervention Investigating the Effects of Acute Exercise on Emotional Regulation. *American Journal of Health Behavior, 41*(5), 534-543.

Ekkekakis, P. (2009). Let them roam free? Physiological and psychological evidence for the potential of self-selected exercise intnsity in public health. *Sports Medicine, 39*, 857-888. doi:https://doi.org/10.2165/11315210-000000000-00000

Ekkekakis, P., Hall, E. E., & Petruzzello, S. J. (1999). Measuring state anxiety in the context of acute exercise using the state anxiety inventory: An attempt to resolve the brouhaha. *Journal of Sport & Exercise Psychology, 21*(205-229).

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). Heart rate variability - Standards of measurement, physiological interpretation, and clinical use. *Circulation, 93*, 1043-1065.

Eley, T. C., Stirling, L., Ehlers, A., Gregory, A. M., & Clark, D. M. (2004). Heart-beat perception, panic/somatic symptoms and anxiety sensitivity in children. *Behav Res Ther, 42*(4), 439-448. doi:10.1016/s0005-7967(03)00152-9

Ensari, I., Greenlee, T. A., Motl, R. W., & Petruzzello, S. J. (2015). Meta-analysis of acute exercise effects on state anxiety: An update of randomized controlled trials over the past 25 years. *Depression and Anxiety, 32*(8), 624-634. doi:10.1002/da.22370

Everson, S. A., Lynch, J. W., Chesney, M. A., Kaplan, G. A., Goldberg, D. E., Shade, S. B., . . . Salonen, J. T. (1997). Interaction of workplace demands and cardiovascular reactivity in progression of carotid atherosclerosis: Population based study. *British Medical Journal, 314*(7080), 553-558.

Executive, H. a. S. (2018). Work related stress depression or anxiety statistics in Great Britain, 2018. Retrieved from http://www.hse.gov.uk/statistics/dayslost.htm

Faravelli, C., & Pallanti, S. (1989). Recent life events and panic disorder. *The American Journal of Psychiatry, 146*(5), 622-626. doi:10.1176/ajp.146.5.622

Feldman, P. J., Cohen, S., Hamrick, N., & Lepore, S. J. (2004). Psychological stress, appraisal, emotion and cardiovascular response in a public speaking task. *Psychology & Health, 19*(3), 353-368. doi:10.1080/0887044042000193497

Feldman, P. J., Cohen, S., Lepore, S. J., Matthews, K. A., Kamarck, T. W., & Marsland, A. L. (1999). Negative emotions and acute physiological responses to stress. *Annals of Behavioral Medicine, 21*(3), 216-222. doi:10.1007/bf02884836

Fischl, B., & Dale, A. M. (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proceedings of the National Academy of Sciences of the United States of America, 97*(20), 11050-11055. doi:http://dx.doi.org/10.1073/pnas.200033797

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., . . . Dale, A. M. (2002). Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron, 33*(3), 341-355. doi:http://dx.doi.org/10.1016/S0896-6273(02)00569-X

Flaa, A., Eide, I. K., Kjeldsen, S. E., & Rostrup, M. (2008). Sympathoadrenal stress reactivity is a predictor of future blood pressure - An 18-year follow-up study. *Hypertension, 52*(2), 336-341. doi:10.1161/hypertensionaha.108.111625

Foss, Ø., & Hallén, J. (2005). Validity and stability of a computerized metabolic system with mixing chamber. *International journal of Sports Medicine, 26*(7), 569-575.

Foundation, M. H. (2018). Mental health statistics: stress. Retrieved from https://www.mentalhealth.org.uk/statistics/mental-health-statistics-stress

Fox, E., Cahill, S., & Zougkou, K. (2010). Preconscious Processing Biases Predict Emotional Reactivity to Stress. *Biological Psychiatry, 67*(4), 371-377. doi:10.1016/j.biopsych.2009.11.0.18

Furay, A. R., Bruestle, A. E., & Herman, J. P. (2008). The Role of the Forebrain Glucocorticoid Receptor in Acute and Chronic Stress. *Endocrinology, 149*(11), 5482-5490. doi:http://dx.doi.org/10.1210/en.2008-0642

Garber, C. E., Blissmer, B., Deschenes, M. R., Franklin, B. A., Lamonte, M. J., Lee, I. M., . . . Swain, D. P. (2011). American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: Guidance for prescribing exercise. *Medicine and science in sports and exercise, 43*(7), 1334-1359.

Gauche, R., Lima, R. M., Myers, J., Gadelha, A. B., Neri, S. G. R., Forjaz, C. L. M., & Vianna, L. C. (2017). Blood pressure reactivity to mental stress is attenuated following resistance exercise in older hypertensive women. *Clinical Interventions in Aging, 12*, 793-802. doi:10.2147/cia.s130787

Gerritsen, L., Kalpouzos, G., Westman, E., Simmons, A., Wahlund, L. O., Backman, L., . . . Wang, H. X. (2015). The influence of negative life events on hippocampal and amygdala volumes in old age: a life-course perspective. *Psychol Med, 45*(6), 1219-1228. doi:10.1017/s0033291714002293

Ghosh, S., Louis, E., Beaugerie, L., Bossuyt, P., Bouguen, G., Bourreille, A., . . . Peyrin-Biroulet, L. (2017). Development of the IBD Disk: A Visual Self-administered Tool for Assessing Disability in Inflammatory Bowel Diseases. *Inflammatory Bowel Diseases, 32*(3), 333-340.

Gianaros, P. J., & Jennings, J. R. (2018). Host in the machine: A neurobiological perspective on stress and cardiovascular disease. *Manuscript submitted for publication*.

Gianaros, P. J., Kuan, D. C., Marsland, A. L., Sheu, L. K., Hackman, D. A., Miller, K. G., & Manuck, S. B. (2017). Community socioeconomic disadvantage in midlife relates to cortical morphology via neuroendocrine and cardiometabolic pathways. *Cerebral Cortex, 27*(1), 460-473. doi:http://dx.doi.org/10.1093/cercor/bhv233

Gianaros, P. J., Onyewuenyi, I. C., Sheu, L. K., Christie, I. C., & Critchley, H. D. (2012). Brain systems for baroreflex supression during stress in humans. *Human Brain Mapping, 33*(7), 1700-1016.

Gianaros, P. J., & Sheu, L. K. (2009). A review of neuroimaging studies of stressor-evoked blood pressure reactivity: Emerging evidence for a brain-body pathway to coronary heart disease risk. *NeuroImage, 47*(3), 922-936. doi:http://dx.doi.org/10.1016/j.neuroimage.2009.04.073

Gianaros, P. J., Sheu, L. K., Matthews, K. A., Jennings, J. R., Manuck, S. B., & Hariri, A. R. (2008). Individual differences in stressor-evoked blood pressure reactivity vary with activation, volume, and functional connectivity of the amygdala. *Journal of Neuroscience, 28*(4), 990-999. doi:http://dx.doi.org/10.1523/JNEUROSCI.3606-07.2008

Gianaros, P. J., Sheu, L. K., Uyar, F., Koushik, J., Jennings, J. R., Wager, T. D., . . . Verstynen, T. D. (2017). A brain phenotype for stressor-evoked blood pressure reactivity. *Journal of the American Heart Association, 6*(9). doi:http://dx.doi.org/10.1161/JAHA.117.006053

Gianaros, P. J., & Wager, T. D. (2015). Brain-Body Pathways Linking Psychological Stress and Physical Health. *Current Directions in Psychological Science, 24*(4), 313-321. doi:http://dx.doi.org/10.1177/0963721415581476

Gilbertson, M. W., Shenton, M. E., Ciszewski, A., Kasai, K., Lasko, N. B., Orr, S. P., & Pitman, R. K. (2002). Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nature Neuroscience, 5*(11), 1242-1247. doi:http://dx.doi.org/10.1038/nn958

Ginty, A. T. (2013). Blunted responses to stress and reward: Reflections on biological disengagement? *International Journal of Psychophysiology, 90*(1), 90-94. doi:10.1016/j.ijpsycho.2013.06.008

Ginty, A. T., Brindle, R. C., & Carroll, D. (2015). Cardiac stress reactions and perseverance: Diminished reactivity is associated with study non-completion. *Biological Psychology, 109*, 200-205. doi:10.1016/j.biopsycho.2015.06.001

Ginty, A. T., Carroll, D., & Williams, S. E. (2014). The relationship between trait anxiety and subjective somatic and actual biological responses to acute psychological stress. *Psychosomatic Medicine, 76*(3), A72-A73.

Ginty, A. T., Gianaros, P. J., Derbyshire, S. W. G., Phillips, A. C., & Carroll, D. (2013). Blunted cardiac stress reactivity relates to neural hypoactivation. *Psychophysiology, 50*(3), 219-229. doi:10.1111/psyp.12017

Ginty, A. T., Kraynak, T. E., Fisher, J. P., & Gianaros, P. J. (2017). Cardiovascular and autonomic reactivity to psychological stress: Neurophysiological substrates and links to cardiovascular disease. *Autonomic Neuroscience, 207*, 2-9. doi:http://dx.doi.org/10.1016/j.autneu.2017.03.003

Glover, G. H. (2011). Overview of Functional Magnetic Resonance Imaging. *Neurosurgery Clinics of North America, 22*(2), 133-139. doi:https://doi.org/10.1016/j.nec.2010.11.001

Golding, L. A., Myers, C. R., & Sinning, W. E. (1989). *Y's way to physical fitness*. Champaign, IL: Human Kinetics.

Gomes, A. R., Faria, S., & Lopes, H. (2016). Stress and Psychological Health: Testing the Mediating Role of Cognitive Appraisal. *Western Journal of Nursing Research, 38*(11), 1448-1468. doi:10.1177/0193945916654666

Gower-Rousseau, C., Sarter, H., Savoye, G., Tavernier, N., Fumery, M., Sandborn, W. J., . . . Peyrin-Biroulet, L. (2017). Validation of the Inflammatory Bowel Disease Disability Index in a population-based cohort. *Gut, 66*(4), 588-596. doi:10.1136/gutjnl-2015-310151

Grimm, O., Pohlack, S., Cacciaglia, R., Winkelmann, T., Plichta, M. M., Demirakca, R., & Flor, H. (2015). Amygdalar and hippocampal volume: A comparison between manual segmentation, Freesurfer and VBM. *Journal of Neuroscience Methods, 253*, 254-261. doi:http://dx.doi.org/10.1016/j.jneumeth.2015.05.024

Gronwall, D. M. A. (1977). Paced auditory serial-addition task - measure of recovery from concussion. *Perceptual and Motor Skills, 44*(2), 367-373. doi:10.2466/pms.1977.44.2.367

Grossman, P., Wilhelm, F. H., Kawachi, I., & Sparrow, D. (2001). Gender differences in psychophysiological responses to speech stress among older social phobics: Congruence and incongruence between self-evaluative and cardiovascular reactions. *Psychosomatic Medicine, 63*(5), 765-777. doi:10.1097/00006842-200109000-00010

Hamer, M., Taylor, A., & Steptoe, A. (2006). The effect of acute aerobic exercise on stress related blood pressure responses: A systematic review and meta-analysis. *Biological Psychology, 71*(2), 183-190. doi:10.1016/j.biopsycho.2005.04.004

Hanin, Y. L. (2000). Individual Zones of Optimal Functioning (IZOF) Model: Emotion-performance relationship in sport. In *Emotions in sport.* (pp. 65-89). Champaign, IL, US: Human Kinetics.

Hanton, S., Mellalieu, S. D., & Hall, R. (2004). Self-confidence and anxiety interpretation: A qualitative investigation. *Psychology of Sport and Exercise, 5*(4), 477-495. doi:10.1016/S1469-0292(03)00040-2

Hassellund, S. S., Flaa, A., Sandvik, L., Kjeldsen, S. E., & Rostrup, M. (2010). Long-term stability of cardiovascular and catecholamine responses to stress tests: an 18-year follow-up study. *Hypertension, 55*, 131-136. doi:http://dx.doi.org/10.1161/HYPERTENSIONAHA.109.143164

Hauser, W., Janke, K. H., Klump, B., & Hinz, A. (2011). Anxiety and depression in patients with inflammatory bowel disease: comparisons with chronic liver disease patients and the general population. *Inflamm Bowel Dis, 17*(2), 621-632. doi:10.1002/ibd.21346

Herd, J., A. (1986). Neuroendocrine mechanisms in coronary heart disease. In K. A. Matthews, S. Weiss, T. Detre, T. M. Dembroski, B. Falkner, S. B. Manuck, & R. Williams (Eds.), *Handbook of stress reactivity and cardiovascular diseases*. New York: Wiley.

Herman, J. P., McKlveen, J. M., Solomon, M. B., Carvalho-Netto, E., & Myers, B. (2012). Neural regulation of the stress response: glucocorticoid feedback mechanisms. *Brazilian Journal of Medical and Biological Research, 45*(4), 292-298. doi:10.1590/s0100-879x2012007500041

Hilmert, C., & Kvasnicka, L. (2010). Blood pressure and emotional responses to stress: Perspectives on cardiovascular reactivity. *Social and personality psychology compass, 4*(7), 470-483.

Hollander, D. (2003). Inflammatory bowel diseases and brain-gut axis. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society, 54 Suppl 4*, 183-190.

Hughes, B. M., Lu, W., & Howard, S. (2018). Cardiovascular stress-response adaptation: Conceptual basis, empirical findings, and implications for disease processes. *Int J Psychophysiol, 131*, 4-12. doi:10.1016/j.ijpsycho.2018.02.003

Hyams, J. S., Fitzgerald, J. E., Treem, W. R., Wyzga, N., & Kreutzer, D. L. (1993). Relationship of functional and antigenic interleukin 6 to disease activity in inflammatory bowel disease. *Gastroenterology, 104*(5), 1285-1292.

Jacobson, L., & Sapolsky, R. (1991). The role of the hippocampus in feedback-regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocrine Reviews, 12*(2), 118-134. doi:http://dx.doi.org/10.1210/edrv-12-2-118

James, W. (1884). What is an Emotion? *Mind, 9*, 188-205.

Jansen, L. M. C., Gispen-de Wied, C. C., & Kahn, R. S. (2000). Selective impairments in the stress response in schizophrenic patients. *Psychopharmacology, 149*(3), 319-325. doi:10.1007/s002130000381

Jennings, J. R., Heim, A. F., Muldoon, M. F., Ryan, C., Gach, H. M., Schirda, C., & Gianaros, P. J. (2017). Brain regional blood flow and working memory performance predict change in blood pressure over 2 years. *Hypertension, 70*(6), 1132-1141. doi:https://doi.org/10.1161/HYPERTENSIONAHA.117.09978

Jennings, J. R., Mendelson, D. N., Muldoon, M. F., Ryan, C. M., Gianaros, P. J., Raz, N., . . . Alzheimers Dis, N. (2012). Regional grey matter shrinks in hypertensive individuals despite successful lowering of blood pressure. *Journal of Human Hypertension, 26*(5), 295-305. doi:http://dx.doi.org/10.1038/jhh.2011.31

Jones, G. (1995). More than just a game - research developments and issues in competitive anxiety in sport. *British Journal of Psychology, 86*, 449-478. doi:10.1111/j.2044-8295.1995.tb02565.x

Jones, G., Hanton, S., & Swain, A. (1994). Intensity and Interpretation of Anxiety Symptoms in Elite and Non-Elite Sports Performers. *Personality and Individual Differences, 17*(5), 657-663. doi:10.1016/0191-8869(94)90138-4

Jones, G., Swain, A., & Hardy, L. (1993). Intensity and Direction Dimensions of Competitive State Anxiety and Relationships with Performance. *Journal of Sport Sciences, 11*(6), 525-532.

Jones, G., & Swain, A. B. J. (1992). Intensity and direction dimensions of competitive state anxiety and relationships with competitiveness. *Perceptual and Motor Skills, 74*, 467-472.

Jones, M., Meijen, C., McCarthy, P. J., & Sheffield, D. (2009). A Theory of Challenge and Threat States in Athletes. *International Review of Sport and Exercise Psychology, 2*(2), 161-280. doi:10.1080/17509840902829331

K. Ko, J., & K. Auyeung, K. (2014). Inflammatory Bowel Disease: Etiology, Pathogenesis and Current Therapy. *Current Pharmaceutical Design, 20*(7), 1082-1096.

Kahn, S., & Khan, R. A. (2017). Chronic Stress Leads to Anxiety and Depression. *Annals of Psychiatry and Mental Health, 5*(1), 1091.

Kamarck, T. W., Debski, T. T., & Manuck, S. B. (2000). Enhancing the laboratory-to-life generalizability of cardiovascular reactivity using multiple occasions of measurement. *Psychophysiology, 37*(4), 533-542. doi:10.1017/s0048577200990644

Kamarck, T. W., Everson, S. A., Kaplan, G. A., Manuck, S. B., Jennings, J. R., Salonen, R., & Salonen, J. T. (1997). Exaggerated blood pressure responses during mental stress are associated with enhanced carotid atherosclerosis in middle-aged Finnish men: findings from the Kuopio Ischemic Heart Disease Study. *Circulation, 96*, 3842-3848.

Kamarck, T. W., Jennings, J. R., Debski, T. T., Glickmanweiss, E., Johnson, P. S., Eddy, M. J., & Manuck, S. B. (1992). Reliable measures of behaviorally-evoked cardiovascular reactivity from a pc-based test battery - results from student and community samples. *Psychophysiology, 29*(1), 17-28. doi:10.1111/j.1469-8986.1992.tb02006.x

Kamarck, T. W., Jennings, J. R., Stewart, C. J., & Eddy, M. J. (1993). Reliable responses to a cardiovascular reactivity protocol: A replication study in a biracial female sample. *Psychophysiology, 30*(6), 627-634. doi:10.1111/j.1469-8986.1993.tb02088.x

Kamarck, T. W., & Lovallo, W. R. (2003). Cardiovascular reactivity to psychological challenge: Conceptual and measurement considerations. *Psychosomatic Medicine, 65*(1), 9-21. doi:10.1097/01.psy.0000030390.34416.3e

Kasprowicz, A. L., Manuck, S. B., Malkoff, S. B., & Krantz, D. S. (1990). Individual differences in behaviorally evoked cardiovascular response: temporal stability and hemodynamic patterning. *Psychophysiology, 27*(6), 605-619. doi:http://dx.doi.org/10.1111/j.1469-8986.1990.tb03181.x

Kaufman, M. P. (2012). The exercise pressor reflex in animals. *Experimental physiology, 97*(1), 51-58.

Keefer, L., Keshavarzian, A., & Mutlu, E. (2008). Reconsidering the methodology of "stress" research in inflammatory bowel disease. *Journal of Crohns & Colitis, 2*(3), 193-201. doi:10.1016/j.crohns.2008.01.002

Kessler, R. C., Berglund, P., Demler, M. A., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry, 62*(6), 593-602. doi:10.1001/archpsyc.62.6.593

King, D., Ellis, K., Seri, S., & Wood, A. (2019). A systematic review of cross-sectional differences and longitudinal changes to the morphometry of the brain following paediatric traumatic brain injury. *23*. doi:10.1016/j.nicl.2019.101844

King, M. G., Burrows, G. D., & Stanley, G. V. (1983). Measurement of stress and arousal: Validation of the stress/arousal adjective checklist. *British Journal of Psychology, 74*(4), 473-479. doi:10.1111/j.2044-8295.1983.tb01880.x

Kivimäki, M., & Steptoe, A. (2018). Effects of stress on the development and progression of cardiovascular disease. *Nature Reviews Cardiology, 15*, 215-229.

Kop, W. J., Weissman, N. J., Zhu, J., Bonsall, R. W., Doyle, M., Stretch, M. R., . . . Tracy, R. P. (2008). Effects of acute mental stress and exercise on inflammatory markers in patients with coronary artery disease and healthy controls. *American Journal of Cardiology, 101*(6), 767-773. doi:10.1016/j.amjcard.2007.11.006

Kozuch, P. L., & Hanauer, S. B. (2008). Treatment of inflammatory bowel disease: a review of medical therapy. *World Journal of Gastroenterology*, *14*(3), 354-377. doi:10.3748/wjg.14.354

Kremen, W. S., Koenen, K. C., Afari, N., & Lyons, M. J. (2012). Twin studies of posttraumatic stress disorder: Differentiating vulnerability factors from sequelae. *Neuropharmacology, 62*(2), 647-653. doi:10.1016/j.neuropharm.2011.03.012

Kuroki, T., Ohta, A., Sherriff-Tadano, R., Matsuura, E., Takashima, T., Iwakiri, R., & Fujimoto, K. (2011). Imbalance in the Stress-Adaptation System in Patients With Inflammatory Bowel Disease. *Biological Research for Nursing*, *13*(4), 391-398. doi:10.1177/1099800410388638

Lakshminarasimhan, H., & Chattarji, S. (2012). Stress Leads to Contrasting Effects on the Levels of Brain Derived Neurotrophic Factor in the Hippocampus and Amygdala. *Plos One*, *7*(1). doi:10.1371/journal.pone.0030481

Lazarus, R. S. (1966). *Psychological stress and the coping process*. New York, NY, US: McGraw-Hill.

Lazarus, R. S. (1999). *Stress and emotion: A new synthesis*. New York: Springer.

Lazarus, R. S., & Folkman, S. (1984). *Stress, Appraisal, and Coping*. New York: Springer.

Levenstein, S. (2003). Stress and ulcerative colitis: Convincing the doubting thomases. *American Journal of Gastroenterology, 98*(10), 2112-2115.

Levenstein, S., Prantera, C., Varvo, V., Scribano, M. L., Berto, E., Luzi, C., & Andreoli, A. (1993). Development of the perceived stress questionnaire - a new tool for psychosomatic research. *Journal of psychosomatic research, 37*(1), 19-32. doi:10.1016/0022-3999(93)90120-5

Linden, W., Rutledge, T., & Con, A. (1998). A case for the usefulness of laboratory social stressors. *Ann Behav Med, 20*(4), 310-316. doi:10.1007/bf02886380

Lindgren, L., Bergdahl, J., & Nyberg, L. (2016). Longitudinal evidence for smaller hippocampus volume as a vulnerability factor for perceived stress. *Cerebral Cortex, 26*(8), 3527-3533. doi:http://dx.doi.org/10.1093/cercor/bhw154

Linninge, C., Jonsson, P., Bolinsson, H., Onning, G., Eriksson, J., Johansson, G., & Ahrne, S. (2018). Effects of acute stress provocation on cortisol levels, zonulin and inflammatory markers in low- and high-stressed men. *Biological Psychology, 138*, 48-55. doi:10.1016/j.biopsycho.2018.08.013

Liu, J. J. W., Ein, N., Gervasio, J., & Vickers, K. (2019). The efficacy of stress reappraisal interventions on stress responsivity: A meta-analysis and systematic review of existing evidence. *Plos One, 14*(2), e0212854-e0212854. doi:10.1371/journal.pone.0212854

Llabre, M. M., Klein, B. R., Saab, P. G., McCalla, J. B., & Schneiderman, N. (1998). Classification of individual differences in cardiovascular responsivity: The contribution of reactor type controlling for race and gender. *International Journal of*

*Behavioral Medicine, 5*(3), 213-229. doi:http://dx.doi.org/10.1207/s15327558ijbm0503_3

Lovallo, W. R. (2005). Cardiovascular reactivity: mechanisms and pathways to cardiovascular disease. *Int J Psychophysiol, 58*(2-3), 119-132. doi:10.1016/j.ijpsycho.2004.11.007

Lovallo, W. R. (2015). *Stress and Health: Biological and psychological interactions*: SAGE Publications, Inc.

Lovallo, W. R., & Gerin, W. (2003). Psychophysiological reactivity: Mechanisms and pathways to cardiovascular disease. *Psychosomatic Medicine, 65*(1), 36-45. doi:10.1097/01.psy.0000033128.44101.c1

Lyons, D. M., Yang, C., Sawyer-Glover, A. M., Moseley, M. E., & Schatzberg, A. F. (2001). Early life stress and inherited variation in monkey hippocampal volumes. *Archives of General Psychiatry, 58*(12), 1145-1151. doi:http://dx.doi.org/10.1001/archpsyc.58.12.1145

Macfarlane, D. J., & Wong, P. (2012). Validity, reliability and stability of the portable Cortex Metamax 3B gas analysis system. *European journal of applied physiology, 112*(7), 2539-2547. doi:http://dx.doi.org/10.1007/s00421-011-2230-7

Macleod, J., Davey Smith, G., Heslop, P., Metcalfe, C., Carroll, D., & Hart, C. (2002). Psychological stress and cardiovascular disease: empirical demonstration of bias in a prospective observational study of Scottish men. *Bmj, 324*(7348), 1247-1251. doi:10.1136/bmj.324.7348.1247

Mallorqui-Bague, N., Bulbena, A., Pailhez, G., Garfinkel, S. N., & Critchley, H. D. (2016). Mind-Body Interactions in Anxiety and Somatic Symptoms. *Harvard Review of Psychiatry, 24*(1), 53-60. doi:10.1097/hrp.0000000000000085

Manuck, S. B., Kasprowicz, A. L., & Muldoon, M. F. (1990). Behaviorally-evoked cardiovascular reactivity and hypertension: Conceptual issues and potential associations. *Annals of Behavioral Medicine, 12*, 17. doi:http://dx.doi.org/10.1207/s15324796abm1201_2

Maroulakis, E., & Zervas, Y. (1993). Effects of aerobic exercise on mood of adult women. *Percept Mot Skills, 76*(3 Pt 1), 795-801. doi:10.2466/pms.1993.76.3.795

Marsland, A. L., Gianaros, P. J., Abrarnowitch, S. M., Manuck, S. B., & Hariri, A. R. (2008). Interleukin-6 covaries inversely with hippocampal grey matter volume in middle-aged adults. *Biological Psychiatry, 64*(6), 484-490. doi:http://dx.doi.org/10.1016/j.biopsych.2008.04.016

Marsland, A. L., Walsh, C., Lockwood, K., & John-Henderson, N. A. (2017). The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. *Brain, Behavior, and Immunity, 64*, 208-219. doi:https://doi.org/10.1016/j.bbi.2017.01.011

Martens, R., Burton, D., Vealey, R. S., Bump, L. A., & Smith, D. E. (1990). Development and validation of the Competitive State Anxiety Inventory-2 (CSAI-2). In R. Martens, R. S. Vealey, & D. Burton (Eds.), *Competitive anxiety in sport* (pp. 193-208). Champaign, IL: Human Kinetics.

Martens, R., Vealey, R. S., & Burton, D. (1990). *Competitive anxiety in sport*. Champaign, IL: Human Kinetics.

Mason, J. W. (1971). A re-evaluation of the concept of "non-specificity" in stress theory. *Journal of psychiatric research, 8*(3-4), 323-333. doi:10.1016/0022-3956(71)90028-8

Mateos-Pérez, J. M., Dadar, M., Lacalle-Aurioles, M., Iturria-Medina, Y., Zeighami, Y., & Evans, A. C. (2018). Structural neuroimaging as clinical predictor: A review of

machine learning applications. *NeuroImage: Clinical, 20*, 506-522. doi:https://doi.org/10.1016/j.nicl.2018.08.019

Maunder, R. G., & Levenstein, S. (2008). The role of stress in the development and clinical course of inflammatory bowel disease: Epidemiological evidence. *Current Molecular Medicine, 8*(4), 247-252. doi:10.2174/156652408784533832

Mauss, I. B., Wilhelm, F. H., & Gross, J. J. (2004). Is there less to social anxiety than meets the eye? Emotion experience, expression, and bodily responding. *Cognition & Emotion, 18*(5), 631-662. doi:10.1080/02699930341000112

Mawdsley, J. E., Macey, M. G., Feakins, R. M., Langmead, L., & Rampton, D. S. (2006). The effect of acute psychologic stress on systemic and rectal mucosal measures of inflammation in ulcerative colitis. *Gastroenterology, 131*(2), 410-419. doi:10.1053/j.gastro.2006.05.017

McArdle, W. D., Katch, F. I., & Katch, V. L. (2001). *Exercise physiology: Energy, nutrition, and human performance*. New York, NY.: Lippincott, Williams, & Wilkins.

McEntee, D. J., & Halgin, R. P. (1999). Cognitive Group Therapy and Aerobic Exercise in the Treatment of Anxiety. *Journal of College Student Psychotherapy, 13*(3), 37-55. doi:10.1300/J035v13n03_04

McEwen, B. S., & Gianaros, P. J. (2011). Stress- and Allostasis-Induced Brain Plasticity. *Annual Review of Medicine, Vol 62, 2011, 62*, 431-445. doi:http://dx.doi.org/10.1146/annurev-med-052209-100430

McGowan, C. R., Robertson, R. J., & Epstein, L. H. (1985). The effect of bicycle ergometer exercise at varying intensities on the heart rate, EMG and mood state responses to a mental arithmetic stressor. *Research Quarterly for Exercise and Sport, 56*, 131-137.

McGregor, H. A., & Elliot, A. J. (2002). Achievement goals as predictors of achievement-relevant processes prior to task engagement. *Journal of educational psychology*, *94*(2), 381-395.

McLean, C. P., Asnaani, A., Litz, B. T., & Hofmann, S. G. (2011). Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. *Journal of psychiatric research*, *45*(8), 1027-1035. doi:10.1016/j.jpsychires.2011.03.006

McNair, D. M. (1984). Manual for the Profile of Mood States. *Current Contents/social & Behavioral Sciences*(27), 20-20.

McNair, D. M., Lorr, M., & Droppleman, L. F. (1971). *Manual for the Profile of Mood States*. Sandiego, CA: Educational and Industrial Testing Services.

Mellalieu, S. D., Hanton, S., & Fletcher, D. (2006). A competitive anxiety review: recent directions in sport psychology research. In S. Hanton & S. D. Mellalieu (Eds.), *Literature reviews in sport psychology* (pp. 4-45). Hauppage, NY: Nova Science.

Meyer-Lindenberg, A., Buckholtz, J. W., Kolachana, B., Hariri, A. R., Pezawas, L., Blasi, G., . . . Weinberger, D. R. (2006). Neural mechanisms of genetic risk for impulsivity and violence in humans. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(16), 6269-6274. doi:http://dx.doi.org/10.1073/pnas.0511311103

Michael, S., Graham, K. S., & Favis, G. M. (2017). Cardiac Autonomic Responses during Exercise and Post-exercise Recovery Using Heart Rate Variability and Systolic Time Intervals—A Review. *Frontiers in Physiology*, *8*, 1-19.

Michielan, A., & D'Inca, R. (2015). Intestinal Permeability in Inflammatory Bowel Disease: Pathogenesis, Clinical Evaluation, and Therapy of Leaky Gut. *Mediators Inflamm, 2015*, 628157. doi:10.1155/2015/628157

Mikocka-Walus, A., Pittet, V., Rossel, J. B., von Kanel, R., & Swiss, I. B. D. C. S. G. (2016). Symptoms of Depression and Anxiety Are Independently Associated With Clinical Recurrence of Inflammatory Bowel Disease. *Clinical Gastroenterology and Hepatology*, *14*(6), 829-+. doi:10.1016/j.cgh.2015.12.045

Mitchell, J. H. (1985). Cardiovascular control during exercise: Central and reflex neural mechanisms. *The American Journal of Cardiology, 55*(10), D34-D41. doi:https://doi.org/10.1016/0002-9149(85)91053-7

Mitsuyama, K., Sata, M., & Tanikawa, K. (1991). Significance of interleukin-6 in patients with inflammatory bowel disease. *Gastroenterol Jpn, 26*(1), 20-28.

Mitsuyama, K., Toyonaga, A., Sasaki, E., Ishida, O., Ikeda, H., Tsuruta, O., . . . Tanikawa, K. (1995). Soluble interleukin-6 receptors in inflammatory bowel disease: relation to circulating interleukin-6. *Gut, 36*(1), 45-49. doi:10.1136/gut.36.1.45

Moore, L. J., Vine, S. J., Wilson, M. R., & Freeman, P. (2012). The effect of challenge and threat states on performance: An examination of potential mechanisms. *Psychophysiology, 49*(10), 1417-1425. doi:10.1111/j.1469-8986.2012.01449.x

Moore, L. J., Wilson, M. R., Vine, S. J., Coussens, A. H., & Freeman, P. (2013). Champ or Chump?: Challenge and Threat States During Pressurized Competition. *Journal of Sport & Exercise Psychology*, *35*(6), 551-562.

Morey, R. A., Petty, C. M., Xu, Y., Hayes, J. P., Wagner, H. R., Lewis, D. V., . . . McCarthy, G. (2009). A comparison of automated segmentation and manual tracing for

quantifying hippocampal and amygdala volumes. *NeuroImage, 45*(3), 855-866. doi:10.1016/j.neuroimage.2008.12.033

Morey, R. A., Selgrade, E. S., Wager, H. R., Huettel, S. A., Wang, L., & McCarthy, G. (2010). Scan-rescan reliability of subcortical brain volumes derived from automated segmentation. *Human Brain Mapping, 31*, 1751-1762. doi:http://dx.doi.org/10.1002/hbm.20973

Morris, L. W., Davis, M. A., & Hutchings, C. H. (1981). Cognitive and emotional components of anxiety: literature review and a revised worry-emotionality scale. *J Educ Psychol, 73*(4), 541-555.

Myers, B. (2017). Corticolimbic regulation of cardiovascular responses to stress. *Physiology & Behavior, 172*, 49-59. doi:http://dx.doi.org/10.1016/j.physbeh.2016.10.015

Myin-Germeys, I., & van Os, J. (2007). Stress-reactivity in psychosis: evidence for an affective pathway to psychosis. *Clin Psychol Rev, 27*(4), 409-424. doi:10.1016/j.cpr.2006.09.005

Nahon, S., Lahmek, P., Durance, C., Olympie, A., Lesgourgues, B., Colombel, J. F., & Gendre, J. P. (2012). Risk factors of anxiety and depression in inflammatory bowel disease. *Inflammatory Bowel Diseases, 18*(11), 2086-2091. doi:10.1002/ibd.22888

Nederend, I., ten Harkel, A. A. D. J., Blom, N. A., Bernston, G. B., & de Geus, E. J. C. (2017). Impedance cardiogaphy in healthy children and chldren with congenital heart disease: Improving stroke volume assessment. *International Journal of Psychophysiology, 120*, 136-147.

Neil, R., Wilson, K., Mellalieu, S. D., Hanton, S., & Taylor, J. (2012). Competitive anxiety intensity and interpretation: A two-study investigation into their relationship with

performance. *International Journal of Sport and Exercise Psychology, 10*(2), 96-111. doi:10.1080/1612197X.2012.645134

Ng, S. C., Shi, H. Y., Hamidi, N., Underwood, F. E., Tang, W., Benchimol, E. I., . . . Kaplan, G. G. (2017). Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *The Lancet, 390*(10114), 2769-2778. doi:https://doi.org/10.1016/S0140-6736(17)32448-0

NICE. (2013). *National Institute for Health and Care Excellence. Ulcerative colitis, management in adults, children and young people* Retrieved from https://www.nice.org.uk/guidance/ng130/evidence/june-2013-full-guideline-pdf-6777262190

Norr, A. M., Albanese, B. J., Allan, N. P., & Schmidt, N. B. (2015). Anxiety sensitivity as a mechanism for gender discrepancies in anxiety and mood symptoms. *Journal of psychiatric research, 62*, 101-107. doi:10.1016/j.jpsychires.2015.01.014

O'Donovan, A., Chao, L. L., Paulson, J., Samuelson, K. W., Shigenaga, J. K., Grunfeld, C., . . . Neylan, T. C. (2015). Altered inflammatory activity associated with reduced hippocampal volume and more severe posttraumatic stress symptoms in Gulf War veterans. *Psychoneuroendocrinology, 51*, 557-566. doi:http://dx.doi.org/10.1016/j.psyneuen.2014.11.010

O'Neill, S. C., Cohen, L. H., Tolpin, L. H., & Gunthert, K. C. (2004). Affective Reactivity to Daily Interpersonal Stressors as a Prospective Predictor of Depressive Symptoms. *Journal of Social and Clinical Psychology, 23*(2), 172-194. doi:10.1521/jscp.23.2.172.31015

Obrist, P. A. (1981). *Cardiovascular psychophysiology: A perspective*. New York: Plenum Press.

Ogden, K. (2012). *Health Psychology: A texbook* (5th ed.). Maidenhead, England: Open University Press.

Olson, C. L. (1976). On choosing a test statistic in MANOVA. *Psychological bulletin, 83*, 579-586.

Oppenheimer, S., & Cechetto, D. (2016). The insular cortex and the regulation of cardiac function. *Comprehensive Physiology, 6*(2), 1081-1133. doi:http://dx.doi.org/10.1002/cphy.c140076

Osadchiy, V., Martin, C. R., & Mayer, E. A. (2019). The Gut-Brain Axis and the Microbiome: Mechanisms and Clinical Implications. *Clin Gastroenterol Hepatol, 17*(2), 322-332. doi:10.1016/j.cgh.2018.10.002

Paine, N. J., Watkins, L. L., Blumenthal, J. A., Kuhn, C. M., & Sherwood, A. (2015). Association of Depressive and Anxiety Symptoms With 24-Hour Urinary Catecholamines in Individuals With Untreated High Blood Pressure. *Psychosomatic Medicine, 77*(2), 136-144. doi:10.1097/psy.0000000000000144

Parrish, B. P., Cohen, L. H., & Laurenceau, J.-P. (2011). Prospective relationship between negative affective reactivity to daily stress and depressive symptoms. *Journal of Social and Clinical Psychology, 30*(3), 270-296. doi:10.1521/jscp.2011.30.3.270

Pavlov, V. A., Wang, H., Czura, C. J., Friedman, S. G., & Tracey, K. J. (2003). The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. *Mol Med, 9*(5-8), 125-134.

Pellissier, S., Dantzer, C., Canini, F., Mathieu, N., & Bonaz, B. (2010). Psychological adjustment and autonomic disturbances in inflammatory bowel diseases and irritable bowel syndrome. *Psychoneuroendocrinology, 35*(5), 653-662. doi:10.1016/j.psyneuen.2009.10.004

Pellissier, S., Dantzer, C., Mondillon, L., Trocme, C., Gauchez, A. S., Ducros, V., . . . Bonaz, B. (2014). Relationship between Vagal Tone, Cortisol, TNF-Alpha, Epinephrine and Negative Affects in Crohn's Disease and Irritable Bowel Syndrome. *Plos One, 9*(9), 9. doi:10.1371/journal.pone.0105328

Penedo, F. J., & Dahn, J. R. (2005). Exercise and well-being: a review of mental and physical health benefits associated with physical activity. *Curr Opin Psychiatry, 18*(2), 189-193.

Perry, N. B., Calkins, S. D., Nelson, J. A., Leerkes, E. M., & Marcovitch, S. (2012). Mothers' responses to children's negative emotions and child emotion regulation: the moderating role of vagal suppression. *Dev Psychobiol, 54*(5), 503-513. doi:10.1002/dev.20608

Peyrin-Biroulet, L., Cieza, A., Sandborn, W. J., Coenen, M., Chowers, Y., Hibi, T., . . . Colombel, J. F. (2012). Development of the first disability index for inflammatory bowel disease based on the international classification of functioning, disability and health. *Gut, 61*(2), 241-247. doi:10.1136/gutjnl-2011-300049

Pezawas, L., Meyer-Lindenberg, A., Drabant, E. M., Verchinski, B. A., Munoz, K. E., Kolachana, B. S., . . . Weinberger, D. R. (2005). 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nature Neuroscience, 8*(6), 828-834. doi:http://dx.doi.org/10.1038/nn1463

Phillips, A. C., Der, G., & Carroll, D. (2009). Self-reported health and cardiovascular reactions to psychological stress in a large community sample: Cross-sectional and prospective associations. *Psychophysiology, 46*(5), 1020-1027. doi:10.1111/j.1469-8986.2009.00843.x

Phillips, A. C., Ginty, A. T., & Hughes, B. M. (2013). The other side of the coin: Blunted cardiovascular and cortisol reactivity are associated with negative health outcomes. *International Journal of Psychophysiology, 90*(1), 1-7. doi:10.1016/j.ijpsycho.2013.02.002

Phillips, A. C., Hunt, K., Der, G., & Carroll, D. (2011). Blunted cardiac reactions to acute psychological stress predict symptoms of depression five years later: Evidence from a large community study. *Psychophysiology, 48*(1), 142-148. doi:10.1111/j.1469-8986.2010.01045.x

Phillips, A. C., Lovallo, W., Jennings, J. R., Ginty, A. T., Salomon, K., & McCubbin, J. (2015). Stress Reactivity and Health: From Exaggerated to Diminished Reactivity, Implications For Disease Risk. *Psychosomatic Medicine, 77*(3), A14-A14.

Pietrzak, R. H., Averill, L. A., Abdallah, C. G., Neumeister, A., Krystal, J. H., Levy, I., & Harpaz-Rotem, I. (2015). Amygdala-Hippocampal Volume and the Phenotypic Heterogeneity of Posttraumatic Stress Disorder: A Cross-Sectional Study. *Jama Psychiatry, 72*(4), 396-398. doi:10.1001/jamapsychiatry.2014.2470

Piwek, L., Ellis, D. A., Andrews, S., & Joinson, A. (2016). The Rise of Consumer Health Wearables: Promises and Barriers. *PLoS Med, 13*(2), e1001953. doi:10.1371/journal.pmed.1001953

Poitras, V. J., & Pyke, K. E. (2013). The impact of acute mental stress on vascular endothelial function: Evidence, mechanisms and importance. *International Journal of Psychophysiology, 88*(2), 124-135. doi:10.1016/j.ijpsycho.2013.03.019

Porges, S. W. (1995). Cardiac vagal tone: a physiological index of stress. *Neurosci Biobehav Rev, 19*(2), 225-233.

Pyke, K. E., & Tschakovsky, M. E. (2005). The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *The Journal of physiology, 568*(Pt 2), 357-369. doi:10.1113/jphysiol.2005.089755

Quigley, K. S., Barrett, L. F., & Weinstein, S. (2002). Cardiovascular patterns associated with threat and challenge appraisals: A within-subjects analysis. *Psychophysiology, 39*(3), 292-302. doi:10.1017/s0048577201393046

Reed, J., & Ones, D. S. (2006). The effect of acute aerobic exercise on positive activated affect: A meta-analysis. *Psychology of Sport and Exercise, 7*(5), 477-514. doi:https://doi.org/10.1016/j.psychsport.2005.11.003

Rejeski, W. J., Hardy, C. J., & Shaw, J. (1991). Psychometric counfounds of assessing state anxiety in conjunction with acute bouts of vigorous exercise. *Journal of Sport & Exercise Psychology, 13*(65-74).

Rejeski, W. J., Thompson, A., Brubaker, P. H., & Miller, H. S. (1992). Acute exercise - buffering psychosocial stress responses in women. *Health Psychology, 11*(6), 355-362. doi:10.1037//0278-6133.11.6.355

Richter, M., Friedrich, A., & Gendolla, G. H. E. (2008). Task difficulty effects on cardiac activity. *Psychophysiology, 45*, 869-875. doi:10.1111/j.1469-8986.2008.00688.x

Ring, C., Burns, V. E., & Carroll, D. (2002). Shifting hemodynamics of blood pressure control during prolonged mental stress. *Psychophysiology, 39*(5), 585-590. doi:10.1111/1469-8986.3950585

Rohleder, N. (2019). Stress and inflammation - The need to address the gap in the transition between acute and chronic stress effects. *Psychoneuroendocrinology, 105*, 164-171. doi:10.1016/j.psyneuen.2019.02.021

Rosenbaum, D. L., White, K. S., & Gervino, E. V. (2012). The impact of perceived stress and perceived control on anxiety and mood disorders in noncardiac chest pain. *J Health Psychol, 17*(8), 1183-1192. doi:10.1177/1359105311433906

Roseth, A. G., Schmidt, P. N., & Fagerhol, M. K. (1999). Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. *Scand J Gastroenterol, 34*(1), 50-54.

Roth, D. L. (1989). Acute emotional and psychophysiological effects of aerobic exercise. *Psychophysiology, 26*(5), 593-602.

Roy, M., & Steptoe, A. (1991). The inhibition of cardiovascular responses to mental stress following aerobic exercise. *Psychophysiology, 28*(6), 689-700.

Rozanski, A., Blumenthal, J. A., & Kaplan, J. (1999). Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation, 99*(16), 2192-2217. doi:10.1161/01.cir.99.16.2192

Saanijoki, T., Nummenmaa, L., Eskelinen, J. J., Savolainen, A. M., Vahlberg, T., Kalliokoski, K. K., & Hannukainen, J. C. (2015). Affective Responses to Repeated Sessions of High-Intensity Interval Training. *Med Sci Sports Exerc, 47*(12), 2604-2611. doi:10.1249/mss.0000000000000721

Sajadinejad, M. S., Asgari, K., Molavi, H., Kalantari, M., & Adibi, P. (2012). Psychological Issues in Inflammatory Bowel Disease: An Overview. *Gastroenterology Research and Practice*. doi:10.1155/2012/106502

Salomon, K., Matthews, K. A., & Allen, M. T. (2000). Patterns of sympathetic and parasympathetic reactivity in a sample of children and adolescents. *Psychophysiology, 37*(6), 842-849. doi:10.1017/s0048577200990607

Sanders, B. J., Wirtz-Nole, C., DeFord, S. M., & Erling, B. F. (1994). Central amygdaloid lesions attenuate cardiovascular responses to acute stress in rats with borderline hypertension. *Physiol Behav, 56*(4), 709-713. doi:10.1016/0031-9384(94)90231-3

Sato, H., & Kawahara, J. (2011). Selective bias in retrospective self-reports of negative mood states. *Anxiety Stress Coping, 24*(4), 359-367. doi:10.1080/10615806.2010.543132

Schachter, S., & Singer, J. (1962). Cognitive, social, and physiological determinants of emotional state. *Psychological review, 69*(5), 379-399.

Schilling, O. K., & Diehl, M. K. (2018). Daily perceptions of control and stress: examining the validity of bidirectional effects. *Innovation in Aging, 2*(Suppl 1), 68-68. doi:10.1093/geroni/igy023.255

Schlotz, W., Hammerfald, K., Ehlert, U., & Gaabb, J. (2011). Individual differences in the cortisol response to stress in young healthy men: Testing the roles of perceived stress reactivity and threat appraisal using multiphase latent growth curve modeling. *Biological Psychology, 87*(2), 257-264. doi:10.1016/j.biopsycho.2011.03.005

Schlotz, W., Kumsta, R., Layes, I., Entringer, S., Jones, A., & Wust, S. (2008). Covariance between psychological and endocrine responses to pharmacological challenge and psychosocial stress: A question of timing. *Psychosomatic Medicine, 70*(7), 787-796. doi:10.1097/PSY.0b013e3181810658

Schmitz, J., Blechert, J., Kramer, M., Asbrand, J., & Tuschen-Caffier, B. (2012). Biased Perception and Interpretation of Bodily Anxiety Symptoms in Childhood Social Anxiety. *Journal of Clinical Child and Adolescent Psychology, 41*(1), 92-102. doi:10.1080/15374416.2012.632349

Schwartz, G. E., Davidson, R. J., & Goleman, D. J. (1978). Patterning of cognitive and somatic processes in self-regulation of anxiety - effects of meditation versus exercise. *Psychosomatic Medicine, 40*(4), 321-328. doi:10.1097/00006842-197806000-00004

Selye, H. (1936). A syndrome produced by diverse nocuous agents. *Nature*(138), 32.

Sexton, K. A., Walker, J. R., Graff, L. A., Bernstein, M. T., Beatie, B., Miller, N., . . . Targownik, L. E. (2017). Evidence of Bidirectional Associations Between Perceived Stress and Symptom Activity: A Prospective Longitudinal Investigation in Inflammatory Bowel Disease. *Inflamm Bowel Dis, 23*(3), 473-483. doi:10.1097/mib.0000000000001040

Sherwood, A., Allen, M. T., Fahrenberg, J., Kelsey, R. M., Lovallo, W. R., & Vandoornen, L. J. P. (1990). Methodological Guidelines for Impedance Cardiography. *Psychophysiology, 27*(1), 1-23.

Sherwood, A., Dolan, C. A., & Light, K. C. (1990). Hemodynamics of blood-pressure responses during active and passive coping. *Psychophysiology, 27*(6), 656-668. doi:http://dx.doi.org/10.1111/j.1469-8986.1990.tb03189.x

Sherwood, A., & Turner, J. R. (1993). Postural stability of hemodynamic-responses during mental challenge. *Psychophysiology, 30*(3), 237-244. doi:http://dx.doi.org/10.1111/j.1469-8986.1993.tb03349.x

Sheu, L. K., Jennings, J. R., & Gianaros, P. J. (2012). Test–retest reliability of an fMRI paradigm for studies of cardiovascular reactivity. *Psychophysiology, 49*, 873-884.

Shiffman, S., Stone, A. A., & Hufford, M. R. (2008). Ecological momentary assessment. *Annu Rev Clin Psychol, 4*, 1-32.

Sibold, J. S., & Berg, K. M. (2010). Mood enhancement persists for up to 12 hours following aerobic exercise: a pilot study. *Percept Mot Skills, 111*(2), 333-342. doi:10.2466/02.06.13.15.pms.111.5.333-342

Siess, J., Blechert, J., & Schmitz, J. (2014). Psychophysiological arousal and biased perception of bodily anxiety symptoms in socially anxious children and adolescents: a systematic review. *European Child & Adolescent Psychiatry, 23*(3), 127-142. doi:10.1007/s00787-013-0443-5

Smith, J. C., O'Connor, P. J., Crabbe, J. B., & Dishman, R. K. (2002). Emotional responsiveness after low- and moderate-intensity exercise and seated rest. *Med Sci Sports Exerc, 34*(7), 1158-1167.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). *STAI: Manual for the State-Trait Anxiety Inventory.* Palo Alto, CA.: Consulting Psychologists Press.

Starkman, M. N., Gebarski, S. S., Berent, S., & Schteingart, D. E. (1992). Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with cushings-syndrome. *Biological Psychiatry, 32*(9), 756-765. doi:https://doi.org/10.1016/0006-3223(92)90079-F

Starkman, M. N., Giodani, B., Gebarski, S. S., Berent, S., Schork, M. A., & Schteingart, D. E. (1999). Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. *Biological Psychiatry, 46*(12), 1595-1602. doi:http://dx.doi.org/10.1016/S0006-3223(99)00203-6

Steptoe, A., Hamer, M., & Chida, Y. (2007). The effects of acute psychological stress on circulating inflammatory factors in humans: A review and meta-analysis. *Brain Behavior and Immunity, 21*(7), 901-912. doi:10.1016/j.bbi.2007.03.011

Steptoe, A., & Kearsley, N. (1990). Cognitive and somatic anxiety. *Behaviour Research and Therapy, 28*(1), 75-81. doi:10.1016/0005-7967(90)90057-p

Steptoe, A., Kearsley, N., & Walters, N. (1993). Cardiovascular activity during mental stress following vigorous exercise in sportsmen and inactive men. *Psychophysiology, 30*(3), 245-252. doi:10.1111/j.1469-8986.1993.tb03350.x

Steptoe, A., & Kivimaki, M. (2012). Stress and cardiovascular disease. *Nat Rev Cardiol, 9*(6), 360-370. doi:10.1038/nrcardio.2012.45

Stewart, S. H., Taylor, S., & Baker, J. M. (1997). Gender differences in dimensions of anxiety sensitivity. *J Anxiety Disord, 11*(2), 179-200. doi:10.1016/s0887-6185(97)00005-4

Steyerberg, E. W., Eijkemans, M. J. C., Harrell, F. E., & Habbema, J. D. F. (2001). Prognostic modeling with logistic regression analysis: In search of a sensible strategy in small data sets. *Medical Descision Making, 21*, 45-56. doi:http://dx.doi.org/10.1177/0272989X0102100106

Studts, J. L. (1995). Short form of the Profile of Mood States (POMS-SF): Psychometric information. *Psychological Assessment, 7*, 80-83.

Stults-Kolehmainen, M. A., & Sinha, R. (2014). The effects of stress on physical activity and exercise. *Sports Med, 44*(1), 81-121. doi:10.1007/s40279-013-0090-5

Swain, A., & Jones, G. (1996). Explaining performance variance: The relative contribution of intensity and direction dimensions of competitive state anxiety. *Anxiety, Stress, & Coping, 9*(1), 1-18. doi:10.1080/10615809608249389

Szabo, A. (2003). Acute psychological benefits of exercise performed at self-selected workloads: Implications for theory and practice. *Journal of Sports Science Medicine, 2*(77-87).

Targownik, L. E., Sexton, K. A., Bernstein, M. T., Beatie, B., Sargent, M., Walker, J. R., & Graff, L. A. (2015). The Relationship Among Perceived Stress, Symptoms, and Inflammation in Persons With Inflammatory Bowel Disease. *American Journal of Gastroenterology, 110*(7), 1001-1012. doi:10.1038/ajg.2015.147

Taylor, S., Jang, K. L., Stewart, S. H., & Stein, M. B. (2008). Etiology of the dimensions of anxiety sensitivity: A behavioral–genetic analysis. *Journal of Anxiety Disorders, 22*(5), 899-914. doi:10.1016/j.janxdis.2007.09.005

Terry, P. C., Lane, A. M., & Fogarty, G. J. (2003). Construct validity of the Profile of Mood States - Adolescents for use with adults. *Psychology of Sport and Exercise, 4*, 125-139.

Thayer, J. F., & Lane, R. D. (2007). The role of vagal function in the risk for cardiovascular disease and mortality. *Biol Psychol, 74*(2), 224-242. doi:10.1016/j.biopsycho.2005.11.013

Thissen, D., Steinberg, L., & Kuang, D. (2002). Quick and easy implementation of the benjamini-hochberg procedure for controlling the false positive rate in multiple comparisons. *Journal of Educational and Behavioral Statistics, 27*(1), 77-83. doi:http://dx.doi.org/10.3102/10769986027001077

Thomas, B. L., Claassen, N., Becker, P., & Viljoen, M. (2019). Validity of Commonly Used Heart Rate Variability Markers of Autonomic Nervous System Function. *Neuropsychobiology, 78*(1), 14-26. doi:10.1159/000495519

Thomas, O., Hanton, S., & Jones, G. (2002). An alternative approach to short-form self-report assessment of competitive anxiety: A research note. *International Journal of Sport Psychology, 33*(3), 325-336.

Tibble, J., Teahon, K., Thjodleifsson, B., Roseth, A., Sigthorsson, G., Bridger, S., . . . Bjarnason, I. (2000). A simple method for assessing intestinal inflammation in Crohn's disease. *Gut, 47*(4), 506-513. doi:10.1136/gut.47.4.506

Triantafillidis, J. K., Merikas, E., & Gikas, A. (2013). Psychological factors and stress in inflammatory bowel disease. *Expert Review of Gastroenterology & Hepatology, 7*(3), 225-238. doi:10.1586/egh.13.4

Trotman, G. P., Gianaros, P. J., Veldhuijzen Van Zanten, J., Williams, S. E., & Ginty, A. T. (2018). Increased stressor-evoked cardiovascular reactivity is associated with reduced amygdala and hippocampus volume. *Psychophysiology, e13277*.

Trotman, G. P., Veldhuijzen van Zanten, J. J. C. S., Davies, J. D., Moller, C., Ginty, A. T., & Williams, S. E. (2019). Associations between heart rate, perceived heart rate, and anxiety during acute psychological stress. *Anxiety Stress and Coping*. doi:10.1080/10615806.2019.1648794

Trotman, G. P., Williams, S. E., Quinton, M. L., & Veldhuijzen Van Zanten, J. J. C. S. (2018). Challenge and threat states: examining cardiovascular, cognitive and affective responses to two distinct laboratory stress tasks. *International Journal of Psychophysiology, 126,* 42-51. doi:10.1016/j.ijpsycho.2018.02.004

Turner, A. I., Smyth, N., Hall, S. J., Torres, S. J., Hussein, M., Jayasinghe, S. U., . . . Clow, A. J. (2019). Reactivity to acute psychological stress and its relationship to future health and disease outcomes: A systematic review of prospective evidence. *Psychosomatic Medicine, 81*(4), A162-A162.

Turner, J. R. (1994). *Cardiovascular Reactivity and Stress*. New York: Plenum Press.

Turner, J. R., Treiber, F. A., Davis, H., Rectanwald, J., Pipkin, W., & Strong, W. B. (1997). Use of a virtual reality car-driving stressor in cardiovascular reactivity research.

*Behavior Research Methods Instruments & Computers, 29*(3), 386-389. doi:10.3758/bf03200591

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience, 10*(6), 397-409. doi:10.1038/nrn2647

Veldhuijzen van Zanten, J. J. C. S., De Boer, D., Harrison, L. K., Ring, C., Carroll, D., Willemsen, G., & De Geus, E. J. C. (2002). Competitiveness and hemodynamic reactions to competition. *Psychophysiology, 39*(6), 759-766. doi:10.1111/1469-8986.3960759

Veldhuijzen Van Zanten, J. J. C. S., Ring, C., Burns, V. E., Edwards, K. M., Drayson, M., & Carroll, D. (2004). Mental stress-induced hemoconcentration: Sex differences and mechanisms. *Psychophysiology, 41*(4), 541-551. doi:10.1111/j.1469-8986.2004.00190.x

Veldhuijzen van Zanten, J. J. C. S., Ring, C., Carroll, D., & Kitas, G. D. (2005). Increased C reactive protein in response to acute stress in patients with rheumatoid arthritis. *Annals of the Rheumatic Diseases, 64*(9), 1299-1304. doi:10.1136/ard.2004.03215

Verma, J. P. (2015). Repeated measures design for empirical researchers. In: John Wiley & Sons.

Wager, T. D., van Ast, V. A., Hughes, B. L., Davidson, M. L., Lindquist, M. A., & Ochsner, K. N. (2009). Brain mediators of cardiovascular responses to social threat, Part II: Prefrontal-subcortical pathways and relationship with anxiety. *NeuroImage, 47*(3), 836-851. doi:http://dx.doi.org/10.1016/j.neuroimage.2009.05.044

Wager, T. D., Waugh, C. E., Lindquist, M., Noll, D. C., Fredrickson, B. L., & Taylor, S. F. (2009). Brain mediators of cardiovascular responses to social threat, Part I: Reciprocal

dorsal and ventral sub-regions of the medial prefrontal cortex and heart-rate reactivity. *NeuroImage, 47*(3), 821-835. doi:https://doi.org/10.1016/j.neuroimage.2009.05.043

Walker, B. R., Best, R., Shackleton, C. H. L., Padfield, P. L., & Edwards, C. R. W. (1996). Increased vasoconstrictor sensitivity to glucocorticoids in essential hypertension. *Hypertension, 27*(2), 190-196. doi:http://dx.doi.org/10.1161/01.HYP.27.2.190

Walker, J. R., Ediger, J. P., Graff, L. A., Greenfeld, J. M., Clara, I., Lix, L., . . . Bernstein, C. N. (2008). The Manitoba IBD cohort study: A population-based study of the prevalence of lifetime and 12-month anxiety and mood disorders. *American Journal of Gastroenterology, 103*(8), 1989-1997. doi:10.1111/j.1572-0241.2008.01980.x

Wang, Q., Verweij, E. W. E., Krugers, H. J., Joels, M., Swaab, D. F., & Lucassen, P. J. (2014). Distribution of the glucocorticoid receptor in the human amygdala; changes in mood disorder patients. *Brain Structure and Function, 219*(5), 1615-1626. doi:http://dx.doi.org/10.1007/s00429-013-0589-4

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and Validation of Brief Measures of Positive and Negative Affect - the Panas Scales. *Journal of Personality and Social Psychology, 54*(6), 1063-1070.

Watson, D., & Pennebaker, J. (1989). Health Complaints, Stress, and Distress: Exploring the Central Role of Negative Affectivity. *Psychological review, 96*, 234-254. doi:10.1037//0033-295X.96.2.234

Wei, L., Hong, C., & Wu, G. (2018). Heart rate variability associated with grey matter volumes in striatal and limbic structures of the central autonomic network. *Brain Research, 1681*, 14-20. doi:http://dx.doi.org/10.1016/j.brainres.2017.12.024

White, R. L., Babic, M. J., Parker, P. D., Lubans, D. R., Astell-Burt, T., & Lonsdale, C. (2017). Domain-Specific Physical Activity and Mental Health: A Meta-analysis. *Am J Prev Med*, *52*(5), 653-666. doi:10.1016/j.amepre.2016.12.008

Whitwell, J. L. (2009). Voxel-based morphometry: an automated technique for assessing structural changes in the brain. *Journal of Neuroscience, 23*(31), 9661-9664. doi:http://dx.doi.org/10.1523/JNEUROSCI.2160-09.2009

Wild, J., Clark, D. M., Ehlers, A., & McManus, F. (2008). Perception of arousal in social anxiety: Effects of false feedback during a social interaction. *Journal of Behavior Therapy and Experimental Psychiatry*, *39*(2), 102-116. doi:10.1016/j.jbtep.2006.11.003

Willemsen, G. H. M., DeGeus, E. J. C., Klaver, C., VanDoornen, L. J. P., & Carroll, D. (1996). Ambulatory monitoring of the impedance cardiogram. *Psychophysiology, 33*(2), 184-193. doi:10.1111/j.1469-8986.1996.tb02122.x

Williams, D. P., Koenig, J., Carnevali, L., Sgoifo, A., Jarczok, M. N., Sternberg, E. M., & Thayer, J. F. (2019). Heart rate variability and inflammation: A meta-analysis of human studies. *Brain, Behavior, and Immunity*, *80*, 219-226. doi:https://doi.org/10.1016/j.bbi.2019.03.009

Williams, S. E., Carroll, D., Veldhuijzen van Zanten, J. J. C. S., & Ginty, A. T. (2016). Anxiety symptom interpretation: A potential mechanism explaining the cardiorespiratory fitness-anxiety relationship. *Journal of Affective Disorders, 193*, 151-156. doi:10.1016/j.jad.2015.12.051

Williams, S. E., Veldhuijzen van Zanten, J. J. C. S., Trotman, G. P., Quinton, M. L., & Ginty, A. T. (2017). Challenge and threat imagery manipulates heart rate and anxiety

responses to stress. *International Journal of Psychophysiology, 117*, 111-118. doi:10.1016/j.ijpsycho.2017.04.011

Williamson, J. W., Fadel, P. J., & Mitchell, J. H. (2006). New insights into central cardiovascular control during exercise in humans: a central command update. *Experimental physiology, 91*(1), 51-58. doi:10.1113/expphysiol.2005.032037

Wright, R. A., Killebrew, K., & Pimpalapure, D. (2002). Cardiovascular incentive effects where a challenge is unfixed: Demonstrations involving social evaluation, evaluator status, and monetary reward. *Psychophysiology, 39*(2), 188-197. doi:10.1017/S0048577201392090

Yang, R. J., Mozhui, K., Karlsson, R. M., Cameron, H. A., Williams, R. W., & Holmes, A. (2008). Variation in mouse basolateral amygdala volume is associated with differences in stress reactivity and fear learning. *Neuropsychopharmacology, 33*(11), 2595-2604. doi:https://doi.org/10.1038/sj.npp.1301665

Yang, X., Daches, S., George, C. J., Kiss, E., Kapornai, K., Baji, I., & Kovacs, M. (2019). Autonomic correlates of lifetime suicidal thoughts and behaviors among adolescents with a history of depression. *Psychophysiology*, e13378. doi:10.1111/psyp.13378

Yang, Y. S., Ryu, G. W., Han, I., Oh, S., & Choi, M. (2018). Ecological Momentary Assessment Using Smartphone-Based Mobile Application for Affect and Stress Assessment. *Healthcare informatics research, 24*(4), 381-386. doi:10.4258/hir.2018.24.4.381

Yerkes, R. M., & Dodson, J. D. (1908). The relationship of strength of stimulus to rapidity of habit formation. *Journal of comparative Neurology and Psychology, 18*, 459-482.

Yeung, R. R. (1996). The acute effects of exercise on mood state. *Journal of psychosomatic research, 40*(2), 123-141. doi:10.1016/0022-3999(95)00554-4

Yılmaz, V., Umay, E., Gündoğdu, İ., Karaahmet, Z. Ö., & Öztürk, A. E. (2017). Rheumatoid Arthritis: Are psychological factors effective in disease flare? *European journal of rheumatology*, *4*(2), 127-132. doi:10.5152/eurjrheum.2017.16100

Zigmond, A. S., & Snaith, R. P. (1983). The hospital anxiety and depression scale. *Acta Psychiatrica Scandinavica, 67*(6), 361-370. doi:10.1111/j.1600-0447.1983.tb09716.x