Measuring the Effects of Stress on Cognitive Function in Real-world and Laboratory Contexts: The Potential Protective Effects of Phospholipid Supplementation

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The candidate confirms that the work submitted is his own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Chapter 4 is partly based on the jointly-authored publication:

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The candidate confirms that he is solely responsible for the collection and analysis of data contained in this publication and wrote the main draft of the paper. The co-authors contributed to the design of the study and provided advice and editing assistance with regards the redrafting of the paper for publication.

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Abstract

There is a well-defined relationship between stress and cognition. The intake of phospholipids has the potential to attenuate responses to stress and thus protect cognitive performance. The aims of this thesis were (a) develop suitable methodologies, both in the real-world and laboratory, to examine the effects of stress on cognition; (b) identify characteristics of individuals who may be particularly stress vulnerable; and (c) assess the potential for a phospholipid intervention to attenuate the response to stress and the impact of this stress exposure on cognitive function. These aims were addressed in four studies. Studies 1 – 3 aimed to identify a number of design and methodological conditions suitable for a phospholipid intervention assessing cognitive performance under stress (Study 4). The identification of a naturalistic or laboratory stress context capable of eliciting cortisol responses over repeated exposures, selection of cognitive tests sensitive to stress impairment, and characterising individuals with an increased tendency towards high cortisol responsivity to stress were the key preparatory methodological factors examined in Studies 1 – 3. Studies 1 (*N* = 16) and 2 (*N* = 17) explored the feasibility of employing a real-world audition stress context for a dietary intervention. Whilst this context was sufficient to elicit significant cortisol responses (Study 1), this was not consistently shown (Study 2), and suggestive of poor test-retest reliability. Study 3 (*N* = 24) demonstrated that a combined physical/psychosocial laboratory stressor was sufficient to elicit significant, and comparable, cortisol responses over repeated exposures. Three tests of prefrontal cortex-mediated cognitive function (n-back, ospan, and task-switch test) were demonstrated to be sensitive to stress impairment (Studies 2 – 3) and the Perfectionism: Organisation dimension of perfectionism emerged as a positive predictor of cortisol responsivity (Studies 1 – 3). The laboratory stressor, identified cognitive tests, and a sample selected on the basis of high perfectionist tendency, were identified as appropriate methodologies for the dietary intervention. These methodologies were employed in a randomised placebo-controlled six week phospholipid intervention (*N* = 54) assessing cognitive performance after an acute physical/psychosocial stressor (pre- and post-intervention). Phospholipid intake was associated with increased cortisol response, and significant elevations in sympathetic and subjective arousal. Cognitive performance was unaffected by phospholipid intake. A trend for attenuated anticipatory subjective stress suggested a modest stress-buffering effect of phospholipids. Further examination of the relationship between perfectionism and cortisol, and determinants of reduced habituation to the laboratory stressor employed, are warranted. The methodological advances developed herein offer a suitable framework for future research further assessing the potential stress-buffering effect of phospholipids in samples characterised by cognitive vulnerabilities (e.g., the elderly).

Publications and Presentations

Publications

Boyle, N. B., Lawton, C., Arkbåge, K., Thorell, L., Dye, L. 2013. Dreading the boards: stress response to a competitive audition characterized by social-evaluative threat. *Anxiety, Stress and Coping: An International Journal,* 26(6), pp 690-699.

Book Chapters

Dye, L., Lamport, D., Boyle. N. B., Hoyland, A. 2011. Macronutrients and cognitive performance. In Benton, D. (Ed.) *Lifetime nutritional influences on cognition, behaviour and psychiatric illness* (pp.435-464). Cambridge, UK: Woodhead Publishing.

Presentations

Boyle, N. B., Lawton, C., Arkbåge, K., Thorell, L., Dye, L. 2009. The potential for milk dairy products and components to alter cognitive performance under stress. University of Leeds Postgraduate Conference, October, 2009.

Boyle, N. B., Lawton, C., Arkbåge, K., Thorell, L., Dye, L. 2010. The validation of a naturalistic stressor characterised by social-evaluative threat: competitive performance auditions as a paradigm for real-world acute psychosocial stress research. UK Society for Behavioural Medicine 6th Annual Meeting, December, 2010.

Boyle, N. B., Lawton, C., Arkbåge, K., Thorell, L., Dye, L. 2012. Impaired Working Memory Performance in the Absence of Cortisol Following a Naturalistic Psychosocial Stressor. British Psychological Society Psychobiological Section Annual Scientific Meeting, September, 2012.

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Symbols and Abbreviations

ACTH	adrenocorticotropic hormone
ANOVA	analysis of variance
AIC	Akaike's Information Criteria
AICC	Akaike's Information Criteria Corrected
AUCg	area under the curve with respect to ground
AUCi	area under the curve with respect to increase
AVP	arginine vasopressin
BBB	blood brain barrier
BC – PS	bovine cortex phosphatidylserine
BIC	Bayesian Information Criteria
BLA	basolateral amygdala
BM-PS	bovine milk-derived phosphatidylserine
BMI	body mass index
BP	blood pressure
CAT	computerised adaptive testing
CAR	cortisol awakening response
CBG	cortisol binding globulin
CNS	central nervous system
CPT	cold pressor test
CRH	corticotrophin-releasing hormone
CSSS	Chronic Stress Screening Scale (TICS)
DBP	diastolic blood pressure
DM	declarative memory
EEG	electroencephalogram

EIA	enzyme immunoassay kit
EOT	External Orientated Thinking (alexithymia)
EPI	epinephrine
ERP	event related potentials
FMPS	Frost Multidimensional Perfectionism Scale
fMRI	functional magnetic resonance imaging
GC	glucocorticoids
GPLs	glycerophospholipids
GRs	glucocorticoid receptors
HADS	Hospital Anxiety (HADS-A) and Depression (HADS-D) Scale
Ho	Cook-Medley Hostility Scale
HPA	hypothalamic-pituitary-adrenal (axis)
IPIP-N	International Personality Item Pool - Neuroticism
LOC	Locus of Control
MAST	Maastricht Acute Stress Test
mg/d	milligrams per day
mmHg	millimetres of mercury
mPFC	medial prefrontal cortex
MRs	mineralocorticoid receptors
ms	milliseconds
NE	norepinephrine
NEO PI-R	Revised NEO Personality Inventory
OC	oral contraceptive
PASA	Primary Appraisal Secondary Appraisal
PAS	phosphatidic acid and PS complex
PFC	prefrontal cortex
PKC	protein kinase C

PL(s)	phospholipid(s)
POMS(-SF)	Profile of Mood States (short form)
PS	phosphatidylserine
PSS	Perceived Stress Scale
PSRS	Perceived Stress Reactivity Scale
PVN	paraventricular nucleus
$\mathbf{r_{\alpha}}$	Chronbach's alpha
$\mathbf{r_{tt}}$	test-retest reliability
RSES	Rosenberg Self-Esteem Scale
RT	reaction time
sAA	salivary α-amylase
SACL	Stress and Arousal Checklist
SAM	sympathetic adrenal-medullary (system)
SBP	systolic blood pressure
SECPT	socially evaluated cold pressor test
SEM	standard error of the mean
SNS	sympathetic nervous system
S-PS	soy- derived phosphatidylserine
SS	sympathoneural system
STAI	Spielberger's State Trait Inventory
TAS-20	Toronto Alexithymia Scale (20-item version)
TICS	Trier Inventory of Chronic Stress
TSST	Trier Social Stress Test
VAS	visual analogue scale
VISGED	Visueller Gedächtnistest (Eng. visual memory test)
WM	working memory
WMC	working memory capacity

Chapter 1:
Literature Review

Chapter 1 Literature Review

The following review of the literature begins with a description of the primary mediators of the psychoneuroendocrine responses to stress provocation; namely the sympathetic nervous system and hypothalamic-pituitary-adrenal axis. These stress response effectors mobilise and regulate the bodily response to challenge and instigate a response cascade that underpins the organism's adaptive reaction to stress. The review will focus upon the HPA axis as this psychoneuroendocrine system, and its endpoint – cortisol, are a primary focus of the aims and objectives of this thesis. Amongst the diverse functions affected by the stress response is cognitive performance. Whilst stress can moderate cognitive processes in an adaptive manner aimed at optimising the perception and processing of the stressor, stress also has the potential to impair cognition function. The review will outline the observed effects of stress on such cognitive processes. Finally, there has been increasing interest in the potential for dietary interventions to moderate aspects of cognitive function. Phospholipids have been associated with the attenuation of endocrine and psychological stress responses and therefore may be considered to offer potential protective effects on cognitive performance under conditions of stress. The review will end with an overview of the current evidence for the potential effects of phospholipids on stress response and cognitive function.

1.1 Stress

A clear consensus on the definition of stress remains elusive. Stress has been used to describe an agent, a context or situation, or a variable that disrupts normal homeostatic functioning. The term is often also used interchangeably to describe the disrupted state; that is, the subsequent effects of disrupted normal functioning. Stress can also be described as acute (singular, intermittent and time-limited), or chronic (continuous and prolonged); processive (predominantly psychological) or physiological in nature (or a combination of both); absolute (universally stressful; e.g., natural disasters) or relative (response mediated by interpretation).

The lack of precision in definition may stem largely from the diverse, yet interconnected, conceptualisations of stress arising from different scientific disciplines. In engineering terms, stress refers to the external strain placed upon a material. Stress may be defined in purely neuroendocrinological terms as any stimulus that provokes the release of catecholamines or glucocorticoids. A social scientist may

define stress as a disturbance of the social milieu in which an individual lives. Physiologists tend to focus upon physical challenges to, and response of, bodily systems (Lazarus & Folkman, 1984). Within psychology, the definition of the term is often influenced by the specific sub-discipline under which it is studied (e.g., cognitive, biological or social). Within the context of this thesis stress and stressor will be used in reference to an agent, context or situation that disrupts or threatens homeostasis. Stress response describes the subjective, behavioural, physiological, and endocrine responses instigated by the stress/stressor.

Stokes and Kite (2001) suggest three traditional models of psychological stress have predominated in stress research – stimulus-based, response-based and transactional models. Stimulus-based models are analogous to engineering models of external strain with an exogenous agent (e.g. workload, noise, temperature) defined as the stressor, and the effects of exposure to this agent considered the consequence of stress. Response-based models emphasise endogenous factors, with stress being defined by the pattern of the behavioural, affective and cognitive response to stressor exposure. These models can be criticised respectively for being overly mechanistic and placing too much emphasis on physiological dimensions. The intra- and inter-individual variability in stress response contradicts simple non-specific activation in favour of transactional models. Transactional models consider the interaction between the individual and the environment with emphasis placed upon the role of individual appraisal (Lazarus & Folkman, 1984; Staal, 2005). Stress is experienced when an individual perceives a mismatch between the demands of a task or context and their ability to cope. Additionally, McGrath (1976) suggests level of motivation and perceived importance of being able to cope with the demand will also contribute to the experience of stress.

Despite numerous definitions, homeostasis is at the core of most conceptions of stress. The maintenance of the equilibrium of homeostasis is the fundamental drive of all living organisms (Chrousos, 2009). When the equilibrium of an organism is threatened, or perceived to be so, by physiological and psychological perturbance from the internal or external environment, physiological, behavioural, and psychological adaptive mechanisms are activated to restore balance. The notion of homeostatic responses restoring critical bodily systems to a stable set point within a narrow range is likely too simplistic and static. In reality, organisms demonstrate complex and dynamic homeostatic set points around which stability is maintained. Allostasis - the maintenance of stability through change - refers to processes that

maintain homeostasis, but emphasises that homeostatic set points change with environmental conditions (McEwen & Seeman, 1999). Allostasis also distinguishes between systems essential for life (homeostasis) and the processes that maintain these systems in balance by responding to physical states (allostasis; e.g., initiation of physiological responses to extremes of temperature [McEwen & Wingfield, 2003]).

The stress response system coordinates the organism's allostatic response to restore homeostasis when challenged by environmental and internal stressors and 'switches off' allostatic responses once the threat has abated (McEwen, 1998a, 1998b). Whilst allostatic responses are adaptive; prolonged, excessive, or repeated exposure to stress can result in a cumulative toll on the body. The allostatic load model (McEwen, 1998a) proposes that such over-activation results in adjusted regulatory 'set points' that ultimately accelerate wear and tear on bodily systems.

Neuroendocrine hormones play a fundamental regulatory role in basal homeostasis and allostatic responses to stress. The principle effectors of the complex repertoire of physiological and behavioural adaptive response to stress are glucocorticoids (GCs) and the catecholamines norepinephrine (NE) and epinephrine (EPI). The psychoneuroendocrine systems underlying this response are outlined below.

1.2 The Psychoneuroendocrine Stress Response

Exposure to an emotionally arousing or stressful stimulus can initiate a variety of physiological responses supporting adaptation to changes in the internal or external environment. The psychoneuroendocrine stress response is primarily mediated by two systems – the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis – which underpin adaptive survival responses characterised by the re-routing of energy resources from non-essential (e.g., appetite, immune and reproductive function) to essential functions related to meeting the challenges of the stressor (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007). Differential reaction patterns to stress have been proposed to distinguish between the two response systems. The SNS response is associated with a swift mobilisation of defence and effort resources required to cope with the immediate challenge of a stressor via the rapid secretion of EPI and NE which act upon key physiological systems associated with the 'fight or flight' response. The HPA system initiates a slower response to stress via the secretion of species-specific glucocorticoids from the adrenal cortex (primarily corticosterone in most rodents; cortisol in humans).

1.2.1 The Sympathetic Nervous System

The efferent arm of the SNS is organised into two major divisions: the sympathetic adrenal-medullary system (SAM) and the sympathoneural system (SS). The SAM is often used to refer to all sympathetic outflow from the efferent arm of the SNS. However, the SS and SAM can be considered anatomically and functionally distinct (Palkovits, 2010). Both divisions of the SNS help to maintain homeostasis after exposure to stress via the outflow of catecholamines from the adrenal medulla and the varicosities of the sympathetic postganglionic axons, instigating adaptive responses to meet the immediate challenge of a stressor. Both divisions of the sympathetic nervous system are mobilised by premotor sympathetic neurons projecting from the forebrain and brain stem (e.g., paraventricular nucleus [PVN], locus coeruleus, and medullary raphe nuclei).

1.2.2 The Sympathetic Adrenal-Medullary System

The SAM system is comprised of sympathetic cholinergic preganglionic neurons and the chromaffin cells of the adrenal medulla. Stimulation of the adrenal medulla by the sympathetic preganglionic neurons instigates the release of EPI and NE into the adrenal drainage system. Catecholamines are released from two types of adrenal chromaffin cells that are expressed in humans at a ratio of approximately 4:1 (EPI-synthesizing:NE-synthesizing). In humans EPI (synonymous with adrenaline) is the predominant catecholamine released from the adrenal medulla (Goldstein, 2010).

Low levels of NE and EPI are released under basal conditions. Circulating levels of catecholamines may increase 10- to 100-fold following exposure to stressors dependent upon stressor intensity and duration (Goldstein, 2012). Under conditions of stress EPI is rapidly dispersed to most cells of the body instigating a number of adaptive responses to cope with the stressor. This includes increased glycogenolysis, metabolic rate, heart rate, bronchodilation and hyperventilation, attention/alertness, and intensification of emotion. Epinephrine also inhibits vegetative functions such as reproduction, digestion and growth (Chrousos, 2009). Epinephrine does not readily enter the brain across the blood brain barrier (BBB). However, systemic adrenaline activates β-adrenoreceptors on vagal afferents that terminate in the nucleus of the solitary tract and locus coereleus. These regions can release NE in the brain (de Kloet, 2000).

1.2.3 The Sympathoneural System

The SS is composed of sympathetic preganglionic neurons and postganglionic neurons that project a postganglionic axon. Preganglionic neurons innervate postganglion neurons via the release of (primarily) acetycholine across the ganglion synapse. Acetylcholine activates nicotinic and muscarinic receptors on postganglionic neurons instigating the release of NE at the axon terminus from varicosities - beaded structures specialised for the storage and release of NE. Norepinephrine is released into the extracellular space via exocytosis and diffuses into local target tissues/organs. A small proportion is drained into blood circulation (Goldstein, 2012).

Norepinephrine is a key neurotransmitter in the control of the cardiovascular system. Once a stressor is encountered, NE released from sympathetic postganglion nerves generally acts to contract smooth muscle fibres triggering vasoconstriction and myocardial contraction. Systemic vasoconstriction increases total peripheral resistance to blood flow in the body. Elevated peripheral resistance and myocardial contraction increases blood pressure which ensures the flow of blood to the vital organs is maintained during stress (Palkovits, 2010).

1.2.4 The Hypothalamic-Pituitary-Adrenal Axis

The pioneering research of Hans Seyle (Seyle, 1936) identified the HPA axis and actions of GCs as primary mediators of the stress response. The HPA axis is a major neuroendocrine pathway comprising the hypothalamus, pituitary gland, and adrenal glands that contributes to maintenance of energy balance and forms a critical part of the endocrine stress response. Parvocellular corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) neurons of the hypothalamus monitor, integrate, and respond to internal or external threats to homeostasis (i.e., stress) relayed via ascending and descending neural pathways. Rat models have indicated inputs from the brain stem and circumventricular afferents, prefrontal cortex (PFC), limbic system (hippocampus, amygdala, and the lateral septum), and raphe nuclei are involved in the activation of the paraventricular nucleus (PVN) under conditions of stress (Burow, Day, & Campeau, 2005; Crane, Buller, & Day, 2003; Day & Akil, 1996; Herman & Cullinan, 1997; Ziegler & Herman, 2002).

In humans, the main peripheral endpoint of the HPA axis is the production of cortisol. Activation of the HPA axis begins with the synthesis and release of CRH and AVP

from the PVN of the hypothalamus. Corticotropin-releasing hormone is released from the median eminence into the portal blood vessels that connect the hypothalamus to the anterior pituitary gland (Nicolson, 2007). Working synergistically with AVP, CRH stimulates the synthesis and release of adrenocorticotropic hormone (ACTH) from the pituitary into systemic circulation. Upon reaching the adrenal cortex, ACTH binds to its adrenocortical cell membranes of the zonae glomerulosa, fasciculate and reticularis and stimulates the synthesis and release of cortisol; principally from the zona fasciculate (see Figure 1.1).

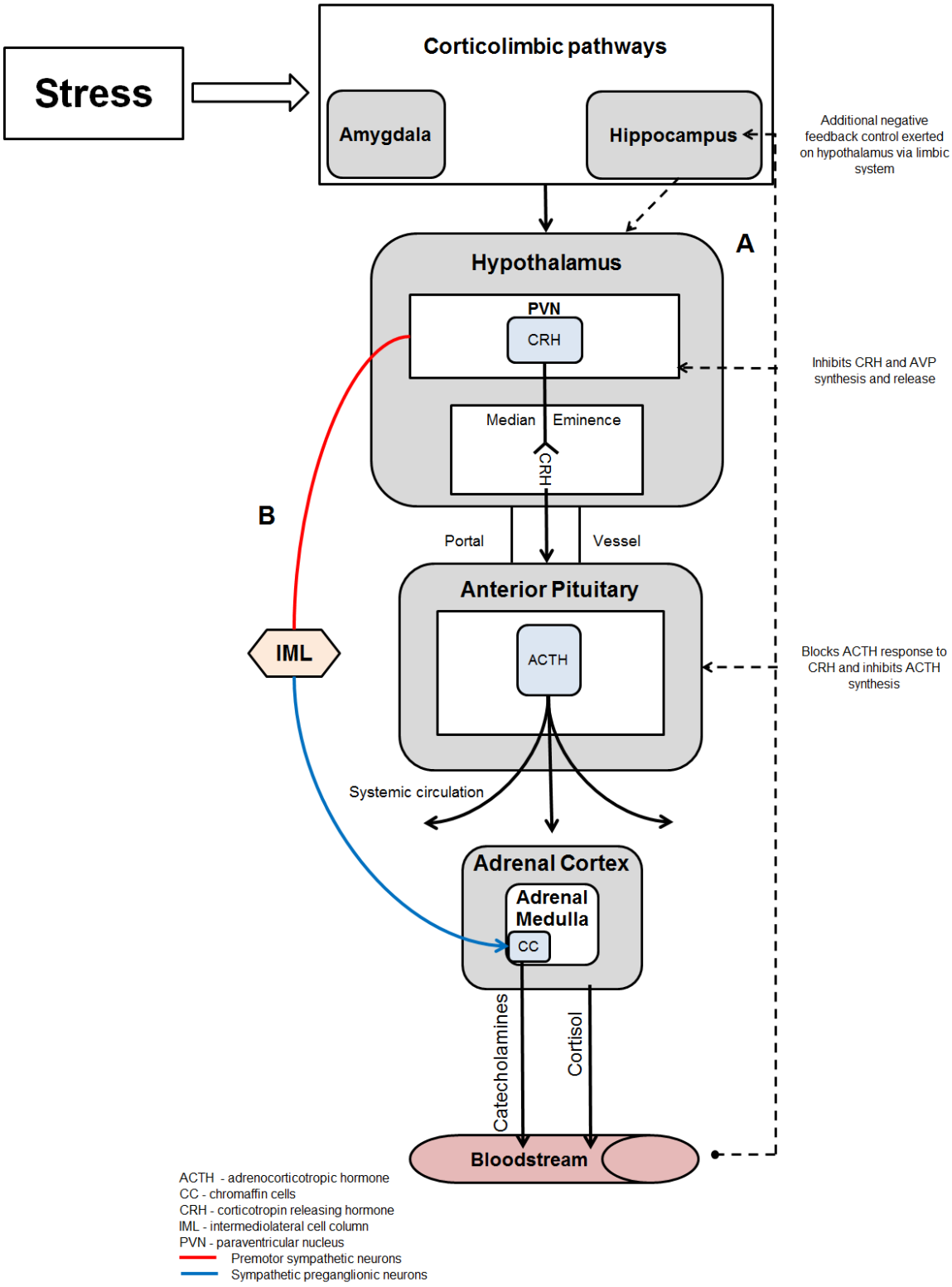


Figure 1.1. Simplified schematic overview of HPA axis (A) and SAM system (B). Dotted lines indicate inhibitory influence; intact lines are excitatory.

1.2.4.1 Cortisol

Cortisol is a small (molecular weight ~362 Dalton) lipophilic steroid. Cortisol is initially unbound following secretion from the adrenal gland into the bloodstream. Approximately 90% of circulating cortisol rapidly binds to specific carrier proteins: cortisol binding globulin (CBG or transcortin), which has a high affinity for cortisol, and a smaller proportion binds to albumin and erythrocytes. It is predominantly the unbound fraction that is proposed to exert its effects on target tissues as unbound cortisol is able to access intercellular GC receptors (Kirschbaum & Hellhammer, 1994; Mendel et al., 1989). However, evidence suggests cortisol bound to carrier proteins may be biologically active to some extent (Hammond, 1995). Specific cell membrane mechanisms that release bound cortisol from carrier proteins also exist in certain tissues. Due to its liposoluble nature, unbound cortisol can easily enter nucleated cells via passive diffusion. Cortisol can also penetrate the BBB (de Kloet, Joels, & Holsboer, 2005; Pardridge & Mietus, 1979).

Cortisol has widespread functional and regulatory effects due to GC receptors being widely distributed throughout the body. Cortisol is known to play a pivotal role in the regulation of most essential physiological processes including homeostatic mechanisms (e.g., metabolic control and immune functioning). Under basal conditions cortisol secretion is controlled within narrow limits and exhibits a circadian rhythm. The HPA axis exhibits a 24 hr circadian profile controlled by the endogenous pacemaker; the suprachiasmatic nucleus. Cortisol secretion is characterised by a circadian peak in the morning (inclusive of the *cortisol awakening response* [CAR] upon waking) and slowly declining levels through the afternoon and evening (circadian trough). Adrenocorticotropic hormone and cortisol are secreted in short pulsatile bursts over the course of the day.

The actions of cortisol are primarily performed intercellularly via genomic mechanisms (slow/nuclear mode of action; Oitzl, Champagne, Van der Veen, & de Kloet, 2010). Accordingly, most of biological responses instigated by cortisol are slow to emerge. However, increasing evidence of rapid glucocorticoid-mediated responses suggests an additional non-genomic action, most likely mediated by GC receptors located on specific cell membranes (fast/membrane mode of action; Groeneweg, Karst, de Kloet, & Joels, 2011).

Two subtypes of receptors mediate the central actions of cortisol: mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs). Glucocorticoid receptors are characterised by differential affinity with MRs binding GCs with an affinity approximately six to ten times that of GRs (de Kloet, Oitzl, & Joels, 1999). As a result of this affinity disparity, occupation of each receptor subtype differs significantly under different conditions and time of day. For example, MRs located in the central nervous system (CNS) may be fully occupied at low physiological cortisol concentrations (cortisol access to MRs is selectively controlled in some tissues [e.g., kidneys]). In contrast, GRs are extensively saturated only during circadian peaks and following exposure to stress.

Activation of the HPA axis under conditions of stress markedly increases cortisol secretion. The increase in circulating cortisol is superimposed against the basal circadian tone. Cortisol instigates both permissive and suppressive responses to stress exposure (Munck & Narayfejestoth, 1992). Permissive effects maintain basal HPA activity and prime stress defence mechanisms to respond to the stressor. Catabolic actions, characterised by increased glycogen storage and gluconeogenesis, metabolism of protein and lipolysis, are initiated in the mobilisation of energy stores. Blood pressure is also raised by multiple steroid mechanisms (Sapolsky, Romero, & Munck, 2000). Cortisol activity is closely linked to central noradrenergic mechanisms, permissively increasing noradrenergic activity during exposure to emotionally arousing stimuli (Roozendaal, Quirarte, & McGaugh, 2002). Cortisol also appears to impinge upon cognitive function and mood, priming the organism's attentional resources and context-dependent memory. The suppressive effects of cortisol underpin stress coping, adaptation, and recovery. Primary mechanisms include anti-inflammatory and immunosuppressive action to prevent damage to the body as a result of defence mechanism overshoot. Cortisol also acts to suppress activity non-essential to meeting the demands of the stressor (e.g., growth and reproductive function). Cortisol exerts permissive influence at low concentrations. Suppressive effects emerge during high cortisol concentrations; following exposure to stress for example. Permissive actions appear to be largely moderated by high affinity MRs, and suppressive actions predominantly by low affinity GRs (Munck & Narayfejestoth, 1992).

Many of the short-term effects of cortisol are essential for survival. Prolonged or repeated excessive exposure to high levels of cortisol can be deleterious. Consequently, activation and inhibition of the response is temporally regulated via a negative endocrine feedback system. Circulating cortisol exerts an inhibitory influence

at the level of the hypothalamus and pituitary gland, inhibiting further release of CRH, AVP and ACTH. Additional negative feedback control is mediated via hippocampal structures acting upon the hypothalamus (Jacobson & Sapolsky, 1991).

1.3 Activation of the Stress Response

Early concepts of stress emphasised the non-specific activation of the HPA axis as a key indicator in determination of a stress response (Seyle, 1936). However, the stress systems, and the HPA in particular, vary in terms of the types of stressful stimuli that activate them. Evidence suggests variability in sympathetic responses to different types of stressors. For example, exposure to cold appears to predominantly activate a SS response as indicated by increased NE. Conversely, the SAM system is predominantly activated when an organism is faced with a psychological stressor, instigating an increased EPI response. Other types of stress activate both sympathetic efferent arms. (Goldstein, 2012; Pacak & Palkovits, 2001; Pacak et al., 1998). An aversive stimulus may activate sympathetic outflow from the adrenal medulla but may not be sufficient to provoke the HPA axis. The HPA axis is not reliably activated under all stress conditions with examples of ostensibly potent stressors (e.g., anticipation of surgical procedures) often evoking only moderate cortisol variations (Biondi & Picardi, 1999).

1.3.1 Psychosocial Determinants of HPA Activation

Research has elucidated the psychological attributes of specific stressful contexts most likely to perturb the hypothalamic-pituitary-adrenal (HPA) axis. Early work identified novelty, unpredictability and uncontrollability as prime candidate characteristics in the elicitation of a significant GC stress response in humans, rodents and non-human primates (Mason, 1968; Rose, 1984). Further evidence for considerable variation in neuroendocrine responses to stress evoking situations in humans has accumulated (Dickerson & Kemeny, 2004; Kudielka, Hellhammer, & Wust, 2009; Singh, Petrides, Gold, Chrousos, & Deuster, 1999). A meta-analytic review of 208 laboratory psychosocial stress studies provides further support for the importance of specific psychological determinants in human cortisol response (Dickerson & Kemeny, 2004). The review findings support a theoretical framework, proposed by Dickerson and Kemeny, that contexts characterised by social-evaluative threat (motivated performance related to an important goal, under conditions of social evaluation and uncontrollability) are prototypical psychological characteristics that

provoke the HPA axis. The level of social-evaluative threat may also moderate sympathetic responses (Bosch et al., 2009).

Concepts of stress response influenced by the motivational perspective propose that a threat or impediment to a central goal is required to activate the HPA axis and mount an adaptive response (Blascovich & Tomaka, 1996; Lazarus & Folkman, 1984). Threat to the central goal of physical self-preservation has been demonstrated to be a key trigger for HPA axis activation in non-human primates (Sapolsky et al., 2000). Influenced by models of primate social behaviour, Dickerson & Kemeny (2004) propose that in addition to the physical self, organisms are motivated to protect and preserve the social self. Threats to the social self – an individual's perception of their own self-worth, social status or value, shaped by the perception and evaluation of others – instigate psychological and physiological responses (the *'social self-preservation system'*) to respond to the threat. Responses proposed to motivate an organism to preserve the social self in the face of threats to social value, esteem, and status include increased negative self-evaluation, and activation of specific behavioural and physiological parameters; including increased cortisol secretion.

Dickerson and Kemeny's review highlighted that motivated public speaking/cognitive laboratory tasks performed in the presence of an evaluative audience with elements of uncontrollability (i.e., behavioural responses cannot affect the outcome) were associated with the largest effect sizes (Cohen's d = .92; Becker, 1988; Dunlap, Cortina, Vaslow, & Burke, 1996). Laboratory stress protocols characterised by conditions of social-evaluative threat, such as the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993) emerged as one of the most reliable methods of inducing stress in a controlled laboratory setting. The TSST is widely considered the gold standard laboratory protocol for experimental psychosocial stress induction with results suggesting the procedure reliably provokes the HPA axis (resulting in an approximately two- to threefold increase in 70-80% of participants; Kudielka, Hellhammer, & Wust, 2009). The TSST requires participants to present themselves as a candidate for a fictitious job (after a 5 – 10 min preparation/anticipation period) and complete a serial subtraction arithmetic task in front of a panel of non-responsive, social-evaluative experimental confederates. The task is characterised by key psychological elements associated with HPA axis activation: completion of a motivated task under conditions of social-evaluative threat and uncontrollability (since the panel are non-responsive regardless of performance level).

The inclusion of the social-evaluative threat element of the TSST appears critical in the elicitation of a cortisol response. Gruenewald, Kemeny, Aziz, & Fahey (2004) compared subjective and endocrine responses to two TSSTs, with and without social-evaluative threat. Only the TSST with a social-evaluative panel elicited a significant cortisol response despite both contexts being subjectively perceived as stressful (indexed by task difficulty and anxiety rating) and eliciting similar cardiovascular responses. Furthermore, significant cortisol responses to a cold pressor test (CPT), a stress induction method comprising immersion of the hand into ice cold water, were *only* apparent when a social-evaluative element was added (socially evaluated cold pressor test [SECPT]; Schwabe & Wolf, 2010). The CPT predominantly activates the SNS resulting in elevated blood pressure (al'Absi, Petersen, & Wittmers, 2002) and increased skin conductance (Buchanan, Tranel, & Adolphs, 2006), but elicits only small or moderate activation of the HPA axis (Al'Absi, Hugdahl, & Lovallo, 2002; Duncko, Johnson, Merikangas, & Grillon, 2009; Gluck, Geliebter, Hung, & Yahav, 2004; McRae et al., 2006). The addition of a social-evaluative confederate and video recording to the SECPT resulted in cortisol responses (comparable to the TSST) significantly greater than those elicited by a traditional CPT and warm water control. No cortisol activation was shown following exposure to a socially evaluated warm water control condition, leading the authors to suggest social evaluation is likely to provoke the HPA axis under conditions in which individuals are concerned about self-presentation (Schwabe & Wolf, 2010).

Comparable variability in cortisol response evident in laboratory stressors is also reported in naturalistic stress contexts (Biondi & Picardi, 1999). A meta-analytic review of cortisol reactivity to naturalistic stress contexts revealed medical (e.g., surgical procedures; Augustin et al., 1999) and sporting performance (e.g., judo contest; Salvador, Suay, Gonzalez-Bono, & Serrano, 2003) stressors to be most efficacious in eliciting a cortisol response (Michaud, Matheson, Kelly, & Anisman, 2008). However, cortisol responses tended to be smaller than those reported in laboratory studies (0 – 180% increase compared to 200 – 300% increase typically reported in TSST studies). Social-evaluative threat may also be an important factor in the provocation of an acute HPA axis stress response in naturalistic contexts. For example, cortisol elevations have been found to be greater during competitive sports performances compared to training at the same level of physical exertion (Cook, Ng, Read, Harris, & Riadfahmy, 1987). Participation in sporting contests may entail elements of social-evaluative threat and uncontrollability (e.g., motivated performance, evaluation by

spectators/teammates and uncontrollable factors such as performance of competitors or injury). However, the relative contribution of elements of social-evaluative threat to cortisol variation in naturalistic settings is often less clear compared to that observed in well-controlled laboratory settings. Social evaluation may not always be present or explicit in sporting contests. Furthermore, the relative contribution of psychosocial factors and physical exertion/sustained training to neuroendocrine response variation is often difficult to disentangle (Michaud et al., 2008).

More explicit examinations of the contribution of social-evaluative threat to cortisol responsivity in naturalistic contexts have been undertaken. Students undertaking a written or oral presentation exam demonstrated a significantly heightened anticipatory (the day before the exam) and post exam elevation in cortisol response for the oral exam (performed in front of examiners) but only moderate anticipatory cortisol elevations for a written exam (Preuss, Schoofs, Schlotz, & Wolf, 2010). Rohleder, Beulen, Chen, Wolf, & Kirschbaum (2007) examined the cortisol responses of individuals taking part in a competitive ballroom dancing contest. Competitive ballroom dancing is characterised by key elements of social-evaluative threat: performance of a valued skill in front of evaluative judges under conditions of uncontrollability (e.g., performance of competitors). This naturalistic context was sufficient to activate cortisol responses that were independent of the physical strain of competition (demonstrated by no cortisol activated to mock competition training). Cortisol responses of greater magnitude were demonstrated when taking part in a dance contest compared to TSST exposure. Moreover, perception of more aspects of the competition as stressful (e.g., the judges and performance satisfaction) was most associated with peak cortisol responses.

1.3.2 Individual Variability in HPA Activation

In addition to the variability in the propensity of stressors to activate the HPA axis, considerable inter-individual variability in cortisol response to stress is commonly observed (Kudielka, Hellhammer, & Wust, 2009). Individual variability in the HPA axis propensity to respond, and magnitude of response, is moderated by a number of demographic, physiological, and biological variables. Cortisol responsivity is influenced by both time-constant (e.g., age, sex, and genetic factors) and time-varying (e.g., smoking and dietary state) factors. A brief overview of selected factors will be outlined here. Several variables will be discussed in relation to the studies presented in this thesis.

Evidence of marginally higher salivary cortisol responses to acute psychosocial stress in older (+ 65 years) vs. younger men (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004), but not young vs. older women (Kudielka, Schmidt-Reinwald, Hellhammer, & Kirschbaum, 1999), has been reported. Sex dimorphism in salivary cortisol responses to acute psychosocial stress is also commonly reported with males typically demonstrating 200 – 400 % response magnitude increase compared to 50 – 150 % in women (Foley & Kirschbaum; Kirschbaum, Wust, & Hellhammer, 1992; Kudielka, Hellhammer, & Wüst, 2009; Kumsta et al., 2007; Lovallo, Farag, Vincent, Thomas, & Wilson, 2006; Seeman, Singer, Wikinson, & McEwen, 2001; Steptoe, Fieldman, Evans, & Perry, 1996). Increased reactivity solely in anticipation of a psychosocial stressor is also often demonstrated only in males (Kirschbaum, Wust, Faig, & Hellhammer, 1992). In addition to the modulating influence of the menstrual cycle and hormonal oral contraception (OC), sex dimorphisms in brain structure and function (Shors, Chua, & Falduto, 2001; Wang et al., 2007), and adrenal cortex sensitivity (Veldhuis et al., 2009) may also play a role in sex-mediated HPA axis reactivity to stress.

Nicotine intake is a powerful stimulator of the HPA axis (Fuxe, Andersson, Eneroth, Harfstrand, & Agnati, 1989; Rosecrans & Karin, 1998). Habitual nicotine intake results in attenuated HPA axis-mediated responses to acute psychosocial stress (Kirschbaum, Scherer, & Strasburger, 1994; Kirschbaum, Strasburger, & Langkrar, 1993), which are not reinstated after abstinence (al'Absi, Amunrud, & Wittmers, 2002). Similarly, chronic alcohol intake may attenuate basal and reactive cortisol responses (Gianoulakis, Dai, & Brown, 2003). However, the evidence is not consistent (Munro, Oswald, Weerts, McCaul, & Wand, 2005).

A number of dietary factors influence the HPA axis. Obesity has been associated with increased HPA axis reactivity to acute psychosocial stress in men (Epel et al., 1999) and women (Epel et al., 2000; Pasquali et al., 1996). This appears specific to central adiposity obesity (indexed by high waist-to-hip ratio). Nutritional status also affects the capacity to mount a cortisol response to acute stress. Long term fasting increases cortisol responsivity to acute stress (Bergendahl, Vance, Iranmanesh, Thorner, & Veldhuis, 1996), whilst short-term fasting abolishes the response (Kirschbaum et al., 1997). Glucose appears to be the key modulator of such effects (discussed further in Section 6.1.4).

Moderate to high heritability of cortisol responsivity to psychosocial stress has been reported (male twin pairs [monozygotic and dizygotic]; Federenko, Nagamine, Hellhammer, Wadhwa, & Wust, 2004). In addition, growing attention is being paid to the identification of genetic polymorphisms underlying HPA axis stress responsivity. Male carriers of the GR gene polymorphisms N363S demonstrate increased responsivity, whilst *Bcl*I carriers are characterised by attenuated responses (Kumsta et al., 2007; Wust et al., 2004). The *Bcl*I GG genotype is associated with high cortisol responses to psychosocial stress in women (Kumsta et al., 2007). Early pre- and post-natal stress and birth-weight have also been associated with lifelong impacts upon HPA axis responsiveness to pharmacological and psychosocial stress (Luecken & Lemery, 2004; Weinstock, 2008; Wust, Entringer, Federenko, Schlotz, & Hellhammer, 2005)

A number of pathological states are associated with attenuated cortisol responses (e.g., chronic fatigue syndrome [Gaab et al., 2002], atopic dermatitis [Buske-Kirschbaum, Geiben, Hollig, Morschhauser, & Hellhammer, 2002], and panic disorder [Petrowski, Herold, Joraschky, Wittchen, & Kirschbaum, 2010]); whilst others are associated with increased responsivity (e.g., major depressive disorder [Burke, Davis, Otte, & Mohr, 2005]). The level of basal stress experienced by individuals is a key modulator of HPA axis responsivity. However, the evidence is heterogeneous with studies reporting both hypo- and hyper-reactivity in individuals experiencing chronic stress (Gaab et al., 2002; Melamed, Shirom, Toker, Berliner, & Shapira, 2006; Tsigos & Chrousos, 1994).

1.3.2.1 Stable predictors of cortisol responsivity

Considering the emphasis given to the individual perception and appraisal of environmental demand in the elicitation of the stress response, it may be suggested that HPA axis responsivity is closely aligned with specific stable personality traits. However, consistent evidence of trait predictors of HPA axis stress responsivity is surprisingly limited (Blood, Blood, Bennett, Simpson, & Susman, 1994; Kirschbaum, Bartussek, & Strasburger, 1992; Salmon et al., 1989; Schommer, Kudielka, Hellhammer, & Kirschbaum, 1999; Schoofs, Hartmann, & Wolf, 2008a; Van Eck, Nicolson, Berkhof, & Sulon, 1996). For example, the reported relationship between the Five Factor Model broad personality dimensions and cortisol responsivity is mixed. Cortisol responsivity has been positively associated with extraversion, and negatively with neuroticism (LeBlanc & Ducharme, 2005; McCleery & Goodwin, 2001; Phillips,

Carroll, Burns, & Drayson, 2005). The relationship between neuroticism and extraversion, and cortisol responsivity may be further moderated by sex (Oswald et al., 2006). Conscientiousness has also been associated with an enhanced cortisol response to stress, while psychoticism was predictive of a blunted response (Garcia-Banda et al., 2011). However, a lack of relationship between broad personality dimensions and cortisol responsivity is often reported (e.g., Arnetz & Fjellner, 1986; Schommer et al., 1999; Wirtz et al., 2007).

Trait anxiety has been shown to be related to dysregulation of the HPA axis. Moderation of the CAR (Schlotz, Hellhammer, Schulz, & Stone, 2004; Walker, O'Connor, Schaefer, Talbot, & Hendrickx, 2011), and diurnal cortisol pattern (Taylor et al., 2008) by trait anxiety has been reported. In terms of cortisol responsivity, it may be predicted that a tendency for high anxiety is associated with increased responses. Indeed, evidence suggests highly anxious individuals may experience a greater number of, and react more negatively to, stressors (Bolger & Zuckerman, 1995). High trait anxiety has been shown to amplify cortisol responses to momentary performance pressure in an ambulatory setting (Schlotz, Schulz, Hellhammer, Stone, & Hellhammer, 2006). However, evidence from individuals experiencing clinical anxiety disturbances is heterogeneous with exaggerated (Condren, O'Neill, Ryan, Barrett, & Thakore, 2002; Dorn et al., 2003; Gerra et al., 2000), attenuated (Petrowski, Wintermann, Schaarschmidt, Bornstein, & Kirschbaum, 2013), and no neuroendocrine stress response reported (Martel et al., 1999). The effect of trait anxiety on cortisol responsiveness in subclinical populations suggests diminished cortisol responsivity in high trait anxious individuals (Beaton et al., 2006; Hubert & Dejongmeyer, 1992; Jezova, Makatsori, Duncko, Moncek, & Jakubek, 2004). It may be hypothesised that increased frequency of reported stress experiences in highly anxious individuals may result in excessive and repeated activation of the HPA axis, resulting in reduced responsivity (Walker et al., 2011). No relationship between cortisol responsivity and trait anxiety has also been reported (Bohnen, Nicolson, Sulon, & Jolles, 1991; Taylor et al., 2008).

More consistent evidence has emerged for the moderation of HPA axis responsivity by self-esteem. Individuals with high self-esteem are characterised by a high level of self-respect, worth, and are appreciative of their own merits. Conversely, low self-esteem denotes a lack of self-respect, worthiness, and feelings of inadequacy (Rosenberg, 1965, 1986). An assumption of the social self-preservation system theory is that physiological responses to social-evaluative threat will be moderated by individual

differences in protective or vulnerability factors (Dickerson & Kemeny, 2004). Ford & Collins (2010) identified self-esteem as a candidate trait factor that may regulate the self-preservation system via a proposed lower threshold for detection of negative social evaluation, and biased negative appraisal of threat, in individuals with low self-esteem. Indeed, Ford and Collins (2010) reported low self-esteem increased negative self-evaluations and HPA axis responsivity to psychosocial stress (interpersonal rejection from an opposite sex partner). Self-esteem has also been demonstrated to be negatively related to the responsivity of the HPA axis response following real-world and pharmacological challenge (Seeman et al., 1995), and a psychosocial (forced failure) stress task (Pruessner, Hellhammer, & Kirschbaum, 1999). The role of self-esteem in the provocation of the HPA axis receives further support from studies demonstrating that retrospective perception of loss of self-esteem following psychosocial stress is associated with magnitude of the elicited cortisol response (Gruenewald et al., 2004).

Stressor controllability has been identified as a prominent psychological factor in the perturbance of the HPA axis (Dickerson & Kemeny, 2004). Locus of control (LOC) orientation may moderate cortisol responsiveness. Locus of control refers to the extent to which individuals perceive events and outcomes to be the result of personal action/behaviour, or factors outside the realm of personal influence (Rotter, 1966). Individuals who perceive events and outcomes in their environment to be derived from personal actions have an internal LOC orientation; individuals who consider outcomes to be largely outside their influence and controlled by environmental factors have an external locus. Bollini, Walker, Hamann, & Kestler (2004) exposed participants to a noise stressor with a varying level of control over noise intensity. Whilst level of control over stressor intensity alone did not predict cortisol response, participants with an internal LOC and high perceived control of the noise stressor demonstrated lower cortisol responsivity.

Further studies have reported a combination of low self-esteem and an external locus of control is associated with a greater propensity toward high cortisol responsivity to psychosocial stress (Pruessner et al., 2005). Furthermore, this propensity may persist over repeated stress exposures (Kirschbaum et al., 1995), and may only fully emerge when responses over repeated stress exposures are aggregated (Pruessner, Gaab et al., 1997).

The role of perfectionism in the elicitation of neuroendocrine responsivity has received some research interest. Traditional models of perfectionism emphasised the setting of excessively high standards as the prominent feature of perfectionism (Pacht, 1984). Frost et al. (2001) considered this tendency to be adjunct to excessive self-critical evaluation of one's own behaviour, a high value attached to parental expectations and evaluations, and an overemphasis on neatness and order. (Frost, Marten, Lahart, & Rosenblate, 1990; Stober, 1998). This multidimensional perfectionist cognitive pattern of excessive standards, self-criticism, and need for order (control) may predispose individuals to increased fear of failure and social-evaluative threat when faced with performing a task in a social context (Flett, Hewitt, Blankstein, & Mosher, 1991; Shafran & Mansell, 2001). Support for this proposition has been reported by Wirtz et al. (2007). Concern over Mistakes and Doubts about Action dimensions of perfectionism were shown to be significant predictors of cortisol (but not EPI or NE) response to a TSST. The authors suggest an excessive fear of failure and negative appraisals of performance that characterise high perfectionists, may be key components of increased HPA axis responsivity to psychosocial stress.

Alexithymia is characterised by a general deficit in understanding, distinguishing, processing, and describing one's own emotions and feelings. High alexithymic individuals also have a propensity to reduce emotional experience and are externally focussed (de Timary, Roy, Luminet, Fille, & Mikolajczak, 2008). Alexithymia has been proposed to be a vulnerability factor for pathology and poor resistance to stress. This 'alexithymia-stress hypothesis' (Martin & Pihl, 1985) has received inconsistent support with some evidence of increased anticipatory autonomic arousal (Berthoz et al., 2002). In terms of cortisol responsivity, an alexithymic dimension (Difficulty Describing Feelings) has been associated with heightened anticipatory, but not reactive, cortisol response to the TSST (de Timary et al., 2008). However, no relationship following psychosocial stress has also been reported (McCaslin et al., 2006).

Trait hostility is a broad psychological domain characterised by negative orientation towards interpersonal interactions. Hostility encompasses negative cognitive, emotional and behavioural responses such as aggression, anger, mistrust, resentment and cynicism (Barefoot, Dodge, Peterson, Dahlstrom, & Williams, 1989). There is some evidence to suggest that individuals characterised by high levels of hostility demonstrate increased and prolonged autonomic and neuroendocrine responses to interpersonally challenging situations (Suarez, Kuhn, Schanberg, Williams, &

Zimmermann, 1998) and marital conflict (Miller, Dopp, Myers, Stevens, & Fahey, 1999).

Finally, there is some evidence to suggest that anticipatory cognitive appraisal processes related to perception, and perceived ability to cope with the demands, of a stressor may be predict cortisol responsivity. This appears logical considering the association between trait variables and stress responsivity is often primarily related to the underlying cognitive patterns or appraisal processes that characterise the personality type (e.g., low self-esteem and negative appraisal of threat). The Primary Appraisal Secondary Appraisal scale (Gaab, Rohleder, Nater, & Ehlert, 2004) and the Perceived Stress Reactivity Scale (Schlotz, Yim, Zoccola, Jansen, & Schulz, 2011) are both measures of anticipatory perceived stress reactivity, and capacity for coping, that have been associated with predictive capacity for heightened HPA axis responses to psychosocial stress (Gaab et al., 2005; Schlotz et al., 2011; Wirtz et al., 2007). These measures are informed by transactional models of stress that emphasise the interaction of the perception of environmental demands and available coping resources in the experience of stress (Lazarus & Folkman, 1984). Therefore an individual's appraisal of the perceived demands of a stressor and coping potential are likely to be predictive of the engendered response.

1.3.3 Habituation of HPA Axis Stress Responses

Rapid habituation of response to stress is a frequently reported characteristic of the HPA axis. Habituation can be defined as a decrement in responsiveness to a stimulus following repeated exposure. In the field of neuroendocrine stress research, habituation usually refers to a reduction in magnitude and/or intensity of the initial psychoneuroendocrine stress response following repeated exposure to an initially novel homotypic stressor (Grissom & Bhatnagar, 2009). Considering the metabolic cost of mounting an acute glucocorticoid response, and the potentially deleterious effects of prolonged exposure, habituation is most likely adaptive. Indeed, a failure to habituate or adjust to repeated exposure to the same stressor underpins the allostatic load state; namely repeated hits, prolonged exposure, inadequate response and lack of adaptation (McEwen, 1998a).

Rodents demonstrate decreased corticosterone responsiveness following repeated restraint (Bhatnagar, Huber, Nowak, & Trotter, 2002; Grissom, Iyer, Vining, & Bhatnagar, 2007), immobilisation (Garcia, Marti, Valles, Dal-Zotto, & Armario, 2000),

and noise stress (De Boer, Van der Gugten, & Slangen, 1989). The cortisol response in humans has been shown to quickly habituate in a number of stress contexts including repeated parachute jumps (Deinzer, Kirschbaum, Gresele, & Hellhammer, 1997) and to psychosocial stress protocols (Federenko et al., 2004; Gerra et al., 2001; Jonsson et al., 2010; Kirschbaum, Pruessner et al., 1995; Schommer, Hellhammer, & Kirschbaum, 2003). Habituation of HPA axis mediated responses has also been shown to increase progressively with each exposure (Deinzer et al., 1997; Gomez, Houshyar, & Dallman, 2002). Habituation in response appears to be specific to the HPA axis. Biomarkers of SAM and SS activation (e.g., EPI, NE, blood pressure [BP]) tend to have comparatively uniform activation patterns across repeated stress exposures (Gerra et al., 2001; Mischler et al., 2005; Schommer et al., 2003; von Kanel, Kudielka, Preckel, Hanebuth, & Fischer, 2006; von Kanel et al., 2004).

A number of psychological characteristics, including novelty, predictability, controllability, and threats to the social-self have been identified as key psychological attributes associated with HPA axis-mediated stress responses (see Section 1.3.1). Repeated exposure to a homotypic stressor is likely to reduce the moderating influence of these psychological characteristics on the engendered response as the contextual and psychological elements of the stressor will be perceived as more familiar, predictable and controllable (Harl, Weisshuhn, & Kerschbaum, 2006; Schommer et al., 2003; Voigt, Ziegler, Grunertfuchs, Bickel, & Fehmwolfsdorf, 1990).

A number of additional factors have been shown to influence habituation of glucocorticoid stress response. Rodent models suggest the HPA axis habituates predominantly to processive (psychological) stressors, and less to physiological (physical) stressors that involve a proximate physical threat (Grissom & Bhatnagar, 2009). Indeed, different neural pathways may underpin HPA axis responses to processive and physiological stressors. Processive stressors primarily activate the PVN via limbic pathways, whilst rapid activation of the PVN via brainstem nuclei, without significant activation of limbic circuitry, has been demonstrated to underpin responses to physical stressors (Emmert & Herman, 1999).

Frequency of stressor exposure is also relevant for HPA axis habituation. In rodents, the more frequent the exposure to stress the more rapid and pronounced the habituation in corticosterone response (Deboer, Koopmans, Slangen, & Van der Gugten, 1990; Ma & Lightman, 1998). A similar effect of frequency may apply to human cortisol habituation but the relationship is less defined. A number of studies

have exposed participants to repeated TSSTs (or modified TSST) separated by intervals of 24 hrs (Epel et al., 2000; Jonsson et al.; Kirschbaum, Pruessner et al., 1995), seven days (Engert et al., 2010; Gerra et al., 2001; von Kanel et al., 2006; Wust, Federenko et al., 2005), and four weeks (Schommer et al., 2003). Overall, mean cortisol responses habituated over repeated exposures. However, Petrowski, Wintermann, and Siepmann (2012) reported that an interval of 10 weeks was sufficient to reinstate previously habituated cortisol responses. Participants were exposed to the TSST on four occasions: time 1 and time 2 separated by 24 hrs; time 3 after an interval of 10 weeks; and time 4 completed 24 hrs after time 3. Cortisol responses to the TSST's habituated when exposures were separated by 24 hrs (time 1 vs. time 2 and time 3 vs. time 4), but no habituation in responses was demonstrated between the TSST's separated by an interval of 10 weeks (time 2 vs. time 3).

The considerable individual variability in HPA axis responses to stress provocation is further reflected in patterns of individual variability in habituation of response. Despite an overall mean pattern of habituation in cortisol response to repeated psychosocial laboratory stressors, a subgroup of non-habituating individuals is often reported. This subgroup usually accounts for approximately one-third of the sample (Gerra et al., 2001; Kirschbaum, Pruessner et al., 1995; Schommer et al., 2003; Wust, Federenko et al., 2005). A smaller proportion of individuals have been shown to demonstrate a sensitisation of response (approximately 16%; Wust et al., 2005). The factors underlying this non-habituation are yet to be fully elucidated. Pruessner et al. (1997) reported that a subset of non-habituators in their study were characterised by a sub-dominance, externally controlled orientation, and insecurity. Kudielka, Bellingrath, & Hellhammer (2006) reported that vital exhaustion, a sequela of chronic stress characterised by high fatigue and loss of energy, was associated with non-habituation in cortisol responses in a small subset of the sample (n = 6). Moreover, an association between heightened cortisol responsivity to psychosocial stress and vital exhaustion is yet to be consistently demonstrated (Kristenson, Kucinskiene, Bergdahl, & Orth-Gomer, 2001; Kristenson et al., 1998; Nicolson & Van Diest, 2000).

Rohleder et al. (2007) reported that the typical pattern of cortisol habituation does not necessarily occur in dancers over repeated exposure to competition stress. No significant differences between cortisol responses over three consecutive competitions or as a function of level of previous competition experience were revealed. The threat of losing social standing related to a skill intimately linked to social status and identity is proposed to underpin this apparent lack of habituation in cortisol response. The

drive to preserve the social self in the face of social-evaluative threat may be so fundamental that, as with threats to the physical self, the cortisol response does not readily habituate. It should be noted that Rohleder et al., were unable to assess responses to competitors first ever competitions, which may well have been even greater in magnitude. However, response decrease towards abolishment over the three competitions would be expected rather than the significant cortisol excursions above baseline levels demonstrated. Further evidence of reduced habituation of cortisol responses to naturalistic stress, more intimately tied to goals and identity was reported by Schoofs, Hartmann, & Wolf (2008b). Salivary cortisol and α-amylase (sAA; a marker of sympathetic activation) responses to a statistical oral examination did not significantly habituate to repeated exposure after three weeks.

1.4 The Effects of Stress on Cognitive Performance

Research interest into the effects of stress on cognition has been primarily shaped by the nature of the physiological response to stress. Cognition, memory in particular, is shaped by stress. Glucocorticoids can directly enter the brain via the BBB where they bind to high-affinity MR and lower-affinity GR receptors inducing rapid non-genomic (Karst et al., 2005), and slower genomic effects (see Section 1.2.4.1; Reul & Dekloet, 1985; Wiegert, Pu, Shor, Joels, & Krugers, 2005). Catecholamines are also released into the brain indirectly via systemic stimulation of the solitary tract and locus coereleus. Neural regions associated with cognitive function are a primary target for adrenal hormones. Interest in the action of GCs on memory function increased following the discovery of GC receptors in neural areas associated with learning and memory in the rodent brain (notably the hippocampus; McEwen, Weiss, & Schwartz, 1968). The two GC receptor subtypes (MRs and GRs) were subsequently identified in the primate brain (Sanchez, Young, Plotsky, & Insel, 2000). Mineralocorticoid receptors are densely expressed in the hippocampal and limbic structures, whilst GRs are also present in these structures, and additionally in the frontal regions (Patel, Katz, Karssen, & Lyons, 2008). A comprehensive review of the experimental literature on the effects of stress on cognitive function is beyond the scope of this thesis. Here, a brief overview of the evidence of the effects of the neuroendocrine stress response on key cognitive domains is provided.

1.4.1 Stress and the Hippocampus

The dense expression of GC receptors in the limbic system has resulted in research into the effects of the neuroendocrine stress response upon cognition being focussed upon hippocampal-dependent memory; namely declarative memory (DM). Declarative memory comprises the process of consciously or voluntarily recollecting previously learned information. In its basic form, DM is composed of three distinct phases: acquisition, consolidation and retrieval. The effect of stress on DM depends critically upon the timing of stress induction in relation to the acquisition, consolidation and retrieval phases of the DM task. Animal and human studies have demonstrated that psychosocial/physical stress or exogenous GC administration in close proximity to acquisition can facilitate subsequent memory (Andreano & Cahill, 2006; Beckner, Tucker, Delville, & Mohr, 2006; Cahill & Alkire, 2003; Roozendaal, Okuda, De Quervain, & McGaligh, 2006; Smeets, Otgaar, Candel, & Wolf, 2008). In contrast, retrieval has been largely shown to be impaired in animals and humans when stress is temporally related to retrieval of previously consolidated material (Buchanan et al., 2006; de Quervain, Roozendaal, & McGaugh, 1998; Kuhlmann, Piel, & Wolf, 2005; Roozendaal, 2003; Tollenaar, Elzinga, Spinhoven, & Everaerd, 2009).

Evidence of the divergent modulating effect of GCs on DM is more consistent in the animal literature. A comparative heterogeneity in human evidence, particularly for memory consolidation (Domes, Heinrichs, Reichwald, & Hautzinger, 2002; Het, Ramlow, & Wolf, 2005; Maheu, Collicutt, Kornik, Moszkowski, & Lupien, 2005), may reflect the challenge of differentiating between acquisition and consolidation processes, with both processes likely to be affected when stress is induced prior to learning. However, despite the difficulty separating the effects of stress induction on DM phase, the evidence supports timing of stress induction in relation to DM phase as a crucial modulatory factor: Hence, memory performance appears to be largely enhanced when stress and GC secretion converge during learning (acquisition and consolidation phases), and impaired when concurrent with retrieval processes (Het et al., 2005; Lupien et al., 2007; Schwabe, Joels, Roozendaal, Wolf, & Oitzl, 2012).

The emotionally arousing quality of the stimuli or stressor employed in studies also appears to moderate stress effects on DM performance. Emotionally arousing material, independent of valence, appears most sensitive to the effects of stress. Greater enhanced consolidation and impaired retrieval of emotionally arousing stimuli (compared to neutral stimuli) has been demonstrated in exogenous GC (Buchanan &

Lovallo, 2001) and psychosocial (Jelicic, Geraerts, Merckelbach, & Guerrieri, 2004) stress studies. This effect appears to require co-occurrence of GCs and noradrenergic activity in the basolateral amygdala (BLA; Roozendaal et al., 2006)

1.4.2 Stress and the Prefrontal Cortex

Glucocorticoid receptors are expressed in moderate to high density in the PFC (Sanchez et al., 2000). The PFC is a target for both circulating GC and NE. Stress-induced cortisol increase has been associated with reduced PFC neural activity (Qin, Hermans, Van Marle, Luo, & Fernandez, 2009). The PFC is also influenced by stress-sensitive noradrenergic projections from the locus coerulues (Schoofs, Preuss, & Wolf, 2008) and catecholamines have been shown to decrease neural firing of PFC neurons via α1 and β1 NE receptors (Ramos & Arnsten, 2007).

The modulation of PFC function by stress hormones is reflected in increasing evidence of PFC-dependent cognition being impaired by high dose administration of GC and psychosocial stress. Working memory (WM) in particular has received increasing research interest. Neuropsychological evidence demonstrates that WM processes predominantly rely upon the integrity of the frontoparietal network (Arnsten, 1998; Jansma, Ramsey, Coppola, & Kahn, 2000; Muller & Knight, 2006; Veltman, Rombouts, & Dolan, 2003). Working memory is a cognitive mechanism that underpins the online processing (e.g., manipulation and updating) and maintenance of information held in memory for a short, temporary period (Baddeley & Hitch, 1974). The revised multi-component model of WM comprises a supervisory (attentional) central executive that controls the sensory input-specific phonological loop, visuo-spatial sketchpad, and episodic buffer subsystems (Baddeley, 1996, 2000).

Acute and short-term pharmacological studies exploring the effects of GCs on cognition suggest WM may be more sensitive to the effects of stress than DM. Young et al. (1999) reported impaired WM performance, but no effects on DM, following corticosterone administration. Similarly, Lupien et al. (1999) demonstrated impaired WM, but not DM, performance following GC infusion. The sensitivity of WM to stress has been supported by studies employing psychosocial stressors (Elzinga and Roelofs, 2005; Schoofs et al., 2008; Luethi et al., 2008; Schoofs et al, 2009), and real-world stress contexts (Klein & Boals, 2001b; Robinson, Sunram-Lea, Leach, & Owen-Lynch, 2008). However, no effect (Hoffman & Al'Absi, 2004; Kuhlmann et al., 2005; Smeets, Jelicic, & Merckelbach, 2006) and enhanced performance (Duncko et al.,

2009) have also been reported. This disparity in effect is likely reflective of WM performance being moderated by the magnitude of the cortisol response elicited. Evidence suggests GCs (and catecholamines) may moderate performance in an inverted-U fashion (discussed further in Section 1.4.3). Indeed, studies reporting no (Hoffman et al., 2004; Smeets et al., 2006) and enhanced (Duncko et al., 2009) WM performance failed to elicit significant cortisol elevations. Another factor that may explain the heterogeneity in the WM literature is the differential sensitivity of specific tests of WM. The level of demand placed upon WM processes by specific tests has been proposed to moderate impairment sensitivity (Elzinga & Roelofs, 2005). For example, more consistent evidence of impaired performance has been reported for WM tests that engage distinguishable working memory processes (e.g., maintenance, manipulation and updating). Furthermore, this is true for both pharmacological GC (Lupien, Gillin, & Hauger, 1999) and psychosocial (Oei, Everaerd, Elzinga, Van Well, & Bermond, 2006; Schoofs et al., 2008) stress studies.

The potential moderation of PFC function by stress hormones has resulted in further investigation of cognitive processes associated with this neural region. Executive function refers to (theorised) cognitive systems that organise, regulate, and control other cognitive processes. Examples include the central executive of the multi-component model of WM (Repovs & Baddeley, 2006) and the supervisory attentional system of attentional control (Shallice, 1982). Executive control is considered to incorporate bottom-up, stimulus driven, and active top-down control processes (e.g., attention, monitoring, planning, and inhibition; Chan, Shum, Toulopoulou, & Chen, 2008; Gruber & Goschke, 2004). Whilst many neural regions are employed in executive control tasks (Alvarez & Emory, 2006), neuroimaging and high-density event-related potential (ERP) studies have identified regions of PFC as central to executive control processes (Mushtaq, Bland, & Schaefer 2013; Wylie, Javitt, & Foxe, 2003)

Executive control is required in situations that involve the rapid and flexible switching between tasks, actions, or goals when cued to do so by environmental demands (Plessow, Kiesel, & Kirschbaum, 2011). Neuropsychological tests measure executive control processes by calculating the cost of switching between tasks (indexed by accuracy and reaction time [RT]) when cued to do so. The cost of switching to a new task (requiring the inhibition of the previous task action) versus the cost of task repetition is considered a measure of cognitive control efficiency (Monsell, 2003). Stress disrupts executive control in rats who show impaired ability to switch between

tasks following stress (Go/noGo paradigm; Butts, Floresco, & Phillips, 2013). Limited studies have explored this effect in stressed humans but available evidence suggests task-switching performance is impaired by psychosocial stress (Plessow et al., 2011; Steinhauser, Maier, & Hubner, 2007).

1.4.3 Potential Mechanisms of the Moderation of Cognitive Function by the Neuroendocrine Stress Response

The potential for GCs to enhance or impair performance may be underpinned by a disparity in GC receptor affinity (de Kloet et al., 1999). Mineralocorticoid receptors bind GCs with an affinity approximately six to ten times that of GR receptors (Reul & Dekloet, 1985). Under basal conditions, human GC secretion exhibits a 24 hr circadian profile. During the evening, 90% of MR and only 10% of GR receptors may be occupied by endogenous GCs. In the morning, or during stress, MR receptors become saturated and GR receptors reach approximately 67-74% saturation levels (Reul & de Kloet, 1985).

Animal models suggest that cortisol-mediated changes in cognitive performance may be largely determined by the ratio of MR and GR saturation (de Kloet et al., 1999). Analogous to the Yerkes-Dodson (Yerkes & Dobson, 1908) inverted U-shaped relationship between arousal and memory, the MR/GR ratio hypothesis suggests cognitive performance can be enhanced under conditions in which the majority of MR and only a small proportion of GR are occupied. Conversely, a significant decrease or increase of GC receptor occupation may result in impaired cognitive performance. Moreover, each GC receptor may operate on distinct yet complimentary stages of cognitive processing. Oitzl and de Kloet (1999) propose that MR play an important role in attention and vigilance, whilst GR are crucial for memory consolidation.

The MR/GR hypothesis receives support from GC removal-replacement studies in which cognitive performance is assessed under conditions of pharmacologically lowered and restored GC levels. Lupien et al. (2002) demonstrated impaired DM following GC depletion was restored following GC replacement. A hydrocortisone infusion study has also shown impaired WM in a dose response, U-shaped function (Lupien et at., 1999). Additional support comes from evidence from psychosocial stress studies that demonstrate impaired cognitive performance only when significant elevations of cortisol are elicited (Buchanan & Tranel, 2008; Elzinga & Roelofs, 2005; Schoofs, Wolf, & Smeets, 2009), and no effect, or enhanced, performance when

elicited cortisol concentrations are low (Andreano & Cahill, 2006; Hoffman & Al'Absi, 2004; Jelicic et al., 2004). Furthermore, the MR/GR ratio hypothesis would predict that time of day, relative to the GC circadian rhythm, would also influence cognitive performance under stress. Stress induced cortisol elevations superimposed on to the morning peak should impair performance more frequently compared to elevations superimposed on to the afternoon trough. A review of studies administering pharmacological GC found support for this hypothesis (Het et al., 2005). Exposure to a psychosocial stressor has also been shown to impair performance in the morning but not afternoon (Maheu et al., 2005). However, this evidence is limited to DM performance and numerous studies have reported impaired performance during the afternoon (e.g., Schoof & Wolf, 1999; Buchanan et al., 2006, 2008; Takahashi et al., 2004), and moderation of cognitive performance independent of time of day (Smeets, 2011; Takahashi et al., 2004).

Stress may modulate cognitive function by inducing a shift in the activity of different brain systems (Schwabe et al., 2012). Animal and human studies have demonstrated that modulation of cognitive functions may require concurrent GC and sympathetic activation of the BLA (Abercrombie, Speck, & Monticelli, 2006; de Quervain, Aerni, & Roozendaal, 2007; Elzinga & Roelofs, 2005; Kuhlmann & Wolf, 2006; Roozendaal & McGaugh, 1997). Noradrenergic activation of the BLA, via the stimulation of the nucleus tractus solitaries/locus coeruleus, appears prerequisite for modulating effects of GC activation on memory. Indeed, animal BLA lesions or β-adrenoreceptor blockade abolish the enhancing effects of GCs on consolidation (Roozendaal & McGaugh, 1997) and retrieval (de Quervain et al., 2007). Human evidence of the effects of GCs on consolidation and retrieval being particularly pronounced for emotionally arousing stimuli (Cahill & Alkire, 2003; Kuhlmann et al., 2005; Roozendaal, 2003; Smeets et al., 2009), and the abolishment of memory modulation effects in a non-arousing test environments (Kuhlmann & Wolf, 2006), further supports this model.

Synergistic GC and noradrenergic activation has been proposed to change the pattern of brain activity in a manner that underpins the differential modulation of memory function (Roozendaal, 2002; Roozendaal, McEwen, & Chattarji, 2009). Concurrent GC and noradrenergic activity is considered to switch the brain into a state that prioritises consolidation at the expense of other memory processes (Roozendaal, Barsegyan, & Lee, 2008; Roozendaal, Hahn, Nathan, de Quervain, & McGaugh, 2004). During this 'memory formation mode' cognitive functions such as attention, encoding, and

consolidation of events relevant to the stressor, are prioritised; whereas competing cognitive operations such as memory retrieval and WM are suppressed (Schwabe et al., 2012). Indeed, animal models have shown the same GC and noradrenergic activation underpinning facilitated memory consolidation impairs retrieval and WM performance (Roozendaal et al., 2008).

1.4.4 Individual Cortisol Responsivity and Cognition

Animal model and human evidence demonstrates that individual differences in cortisol (and noradrenergic) activation following stress exposure modulate cognitive function (Elzinga & Roelofs, 2005; McIntyre, Hatfield, & McGaugh, 2002; Tuinstra et al., 2000).The inter-individual variability in cortisol response commonly demonstrated by research participants exposed to stressors has been proposed to be a contributory factor in the modulation of cognitive function under conditions of stress. Whilst some participants demonstrate large cortisol elevations to minimally stressful stimuli, others are characterised by minimal or an absence of cortisol responsivity to potent stress exposure. In addition, heterogeneity in cortisol response to stress has been demonstrated in both laboratory-based and real-world studies (Buchanan et al., 2006; Harl et al., 2006; Martinek, Oberascher-Holzinger, Weishuhn, Klimesch, & Kerschbaum, 2003; Takahashi et al., 2004). This variability in cortisol responsivity may impact upon subsequent modulation of cognitive function. For example, the impairment of WM appears to require significant increases in cortisol concentrations. Pharmacological studies have demonstrated a corticosterone administration dose-response effect with WM impairment under high doses (Lupien et al., 1999; Wolf, Convit et al., 2001), whilst no effect/trends towards improvement have been found at low doses (Lupien et al., 1999; Monk & Nelson, 2002). Variability in cortisol response has also been shown to have moderating effects in other domains (e.g., increased post-stress snack intake only in high cortisol responders; Newman, O'Connor, & Conner, 2007)

High variability in HPA axis responsivity to psychosocial stress provocation often results in the post-hoc split of participants who demonstrate a high or low cortisol response. Cognitive performance outcomes are subsequently compared across the responder and non-responder groups. This split is commonly made based upon observation of individual cortisol response profiles to stress or by comparing aggregated measures of cortisol. For example, a post-hoc median split based upon the absolute difference between peak post-stress cortisol and baseline cortisol levels

(delta increase) or area under the curve (AUC) aggregations (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

A number of studies have reported moderating effects of psychosocial stress on cognitive performance only in individuals demonstrating large cortisol responses. This selective moderation has been shown across a number of cognitive domains including DM (Buchanan & Tranel, 2008; Buchanan et al., 2006; Nater et al., 2007; Wolf, Schommer, Hellhammer, McEwen, & Kirschbaum, 2001), social memory (memory of faces; Takahashi et al., 2004), and WM (Elzinga & Roelofs, 2005). Adrenergic activation again emerged as an important factor in the observation of these effects. For example, Buchanan et al. (2006) demonstrated impaired DM retrieval in high cortisol responders compared to low responders and non-stress controls. This effect was most pronounced for arousing stimuli (words) emphasising a role for concurrent cortisol and sympathetic arousal. Similarly, Elzinga and Roelofs (2005) reported that whilst cortisol increase was the only significant independent predictor of impaired WM, impaired performance was only shown by a subset of high cortisol responders during enhanced (adrenergic) arousal. Increased cortisol responsivity was the only factor that distinguished high and low stress responders (homogeneous across sympathetic and subjective response). Also, performance in high cortisol responders was indistinguishable from non-responders once sympathetic arousal had abated.

1.4.5 Summary of the Effects of Stress on Cognition

The effects of stress on cognition are proposed to be underpinned by the hormones and neurotransmitters of two interacting psychoneuroendocrine systems: the SAM/SS and the HPA axis. The key role of GCs and catecholamines have been emphasised as these hormones can modulate activity in noradrenergic and GC receptors in key neural regions underpinning cognitive function. Research suggests the effects of stress on memory performance are largely dependent upon a number of modulatory variables specific to each memory type. Evidence has demonstrated that stress can have an impairing or enhancing effect upon DM depending upon the proximity of stress to specific cognitive processes (e.g., consolidation or retrieval). Cognitive processes dependent upon the PFC appear to be more sensitive to impairment but have been under-researched.

The potential for stress to moderate cognitive performance appears to be influenced by the magnitude of the cortisol response elicited. This moderation of performance

may operate in an inverted-U function. High concentrations of cortisol alone appear sufficient to modulate cognitive function (e.g., the impairment of WM by high dose corticosterone; Lupien et al., 1999; Hsu et al., 2003). However, the concentrations of cortisol elicited by mild psychosocial stressors may require concurrent sympathetic (adrenergic) arousal to impact upon cognitive performance. The interaction of stress hormones is proposed to induce an adaptive shift in cognitive processing that prioritises cognitive functions needed to attend and respond to a stressor (e.g., attention and memory consolidation) at the expense of other cognitive processes (e.g., memory retrieval and WM).

1.5 The Potential for Phospholipids to Moderate Cognitive Performance under Stress

There has been widespread research interest into the potential for macro- and micronutrients to impact upon cognitive performance. Food components are proposed to alter cognitive function by exerting effects on neural cell structure, neurotransmission, energy supply to the brain, and metabolism (Dye, Lluch, & Blundell, 2000; Schmitt, Benton, & Kallus, 2005). Increasing interest has been shown in the potential for milk dairy products and components to impact upon cognitive functions. Evidence of stress-reducing effects of phospholipids (PLs) raises the potential hypothesis that PL supplementation may offer protective effects on cognition under conditions of stress.

1.5.1 Phospholipids

Phospholipids are a class of lipids essential to the lipid bilayer of cell membranes. A hydrophobic tail and a hydrophilic head give PLs amphiphilic properties that underpin their important role in the structural and physical integrity of natural membranes. Glycerophospholipids (GPLs) are the most common PLs in cell membranes. Glycerophospholipids comprise fatty acids esterified to a glycerol backbone, a phosphate group, and a hydrophilic residue. Phospholipids with a long chain amino-alcohol sphingosin backbone (instead of glycerol) are classified as sphingophospholipids (Kuellenberg, Taylor, Schneider, & Massing, 2012). Phospholipids are provided directly from dietary intake or via de novo synthesis. Daily consumption of PLs is typically low (approximately 2 - 5 g per day; Fave, Coste, &

Armand, 2004). The main dietary sources of PLs are eggs, soybeans, meat, milk and other dairy products (MacKenzie, Vyssotski, & Nekrasov, 2009).

Glycerophospholipids are extensively (> 90%) absorbed in the intestines, predominantly hydrolysed by phospholipase A_2, and taken up by enterocytes as free fatty acids and lysophospholipids (Kuellenberg et al., 2012). Both lipids can be re-esterified to GPLs and enter the bloodstream. A small proportion of PLs may also be passively absorbed without hydrolysation and incorporated into high density lipoproteins (Zierenberg & Grundy, 1982). The mechanisms via which PLs are incorporated into cellular membranes are complex and not fully characterised. However, dietary PL fatty acids are incorporated into cellular membranes altering cell composition. It should be noted that evidence of the action of dietary PLs at the cellular level is predominantly from *in vitro* analysis. For example, evidence from animal cell studies has demonstrated the efficient incorporation of the GPL phosphatidylserine (PS) into cell cultures (Nishijima, Kuge, & Akamatsu, 1986; Taniguchi, Kashiwayanagi, & Kurihara, 1994), and brain synaptosomes (Floreani, Debetto, & Carpenedo, 1991). Cell membrane action of PLs *in vivo*, particularly in humans, is poorly characterised due to the complexity of the analysis (Kuellenberg et al., 2012).

Bovine milk-derived PLs are the focus of this thesis. Phospholipids account for approximately 0.2 – 1.0 wt % of total bovine milk lipids (Molkentin, 1999). Together with proteins, PLs comprise the main constituents of the milk fat globule membrane (Contarini & Povolo, 2013). Glycerophospholipids and sphingolipids are the most abundant phospholipids in milk. These are principally phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol and sphingomyelin. The composition of bovine milk PLs is shown in Table 1.1.

Table 1.1 Approximate percentage of Phospholipid fractions in bovine milk

Lipid class	Approx. % of total phospholipid fraction
Phosphatidylethanolamine	27%
Phosphatidylcholine	27%
Sphingomyelin	25%
Phosphatidylserine	13%
Phosphatidylinositol	7%
Other	1%

1.5.2 Potential Functional Properties of Phospholipids

The key physiological properties and functions of PLs have resulted in research interest examining the potential functional benefits of PL intake. Phospholipids perform a variety of cell membrane structural and regulatory functions. Research has predominantly focussed upon PS due to its key role in neuronal cell structure and functioning (McDaniel, Maier, & Einstein, 2003). Phosphatidylserine is a relatively minor acidic membrane PL accounting for 2-10% of total PLs in the cell membrane bilayer of mammalian cells (Vance & Steenbergen, 2005). In humans PS is most concentrated in the brain where it accounts for approximately 15% of the PL pool (Baumeister, Barthel, Geiss, & Weiss, 2008).

Rat models have demonstrated that PS plays an important role in the functioning of neuron cell membranes. Phosphatidylserine is crucial in the determination of the surface potential of neuronal membranes which is essential for intercellular communication. The presence of PS within the neuronal membrane is also crucial for the action of protein kinase C (PKC). Protein kinase C is an intracellular messenger enzyme that regulates the release of neurotransmitters implicated in learning and memory (e.g., acetylcholine and dopamine; Blokland, Honing, Brouns, Jolles, 1999; McDaniel et al., 2004; Mochizuki, 1984).

The specific functions of PS in neuronal cells stimulated research which examined the potential for this nutrient to protect cognitive function. The composition of neuronal membranes changes as a result of the ageing process. Such changes affect membrane fluidity, potentially affecting the release of neuronal neurotransmitters and impairing intracellular signalling. Early animal models suggest PS may attenuate this age-related deterioration of neuronal membranes and functioning. Long-term supplementation of PS has been shown to attenuate age-associated dendritic spine

loss (Nunzi, Milan, Guidolin, & Toffano, 1987), counteract the reduced release of neurotransmitters associated with ageing (e.g., acetylcholine; Casamenti, Scali, & Pepeu, 1991), and attenuate age-associated cholinergic hypofunction (Pedata, Giovannelli, Spignoli, Giovannini, & Pepeu, 1985). Such effects of PS supplementation on neuronal functioning have been proposed to underpin behavioural evidence of improved cognitive performance in aged rats after intraperitoneal injection (Aporti et al., 1986; Calderini et al., 1985; Drago, Canonico, & Scapagnini, 1981), and oral administration (Zanotti, Valzelli, & Toffano, 1989).

The potential for this nutrient to modulate age-associated and dementia–related cognitive impairment in humans has also been examined. Evidence of the potential for PS to protect cognitive function in individuals with early onset dementia (Crook, Petrie, Wells, & Massari, 1992; Delwaide, Gyselynckmambourg, Hurlet, & Ylieff, 1986) and non-dementia related age-associated memory decline (Cenacchi et al., 1993; Crook et al., 1991) has been demonstrated. However, such findings tend to be modest and inconsistent (Jorissen et al., 2001).

1.5.3 Phospholipids and Stress

Evidence has suggested that PS and enriched PL drinks (containing PS) may offer potential stress-buffering effects via the attenuation of HPA axis-mediated responses to stress.

1.5.3.1 Early Evidence

Early research examining the functional potential of PS focussed on this phospholipid extracted from the bovine cortex. Bovine cortex phosphatidylserine (BC-PS) has demonstrated stress-buffering effects on stress-induced activation of the HPA axis in males. Monteleone, Maj, Beinat, Natale, & Kemali (1992) reported that a ten day 800 mg/d intake of PS attenuated plasma ACTH and cortisol, compared to a placebo and 400 mg/d dose, following acute exhaustive bicycle ergometer exercise. This study replicated the findings of an earlier study by the authors that reported attenuated plasma cortisol and ACTH following intravenous administration of 50 and 75 mg of BC-PS (Monteleone, Beinat, Tanzillo, Maj, & Kemali, 1990).

1.5.3.2 Recent Evidence

Extraction of PS from bovine cortex is now considered problematic due to the recognition of the potential transfer of infectious disease (e.g., bovine spongiform encephalopathy). More recent studies examining the effects of PS on parameters of stress have administered PS extracted from alternative sources. The most common forms of PS are derived from soy (S-PS) and bovine milk (BM-PS). Evidence of the stress-buffering effects of PS derived from these nutrient sources is heterogeneous. However, there is some support for the potential of this nutrient to modulate stress responses.

The potential for PS to attenuate cortisol responses to exhaustive exercise stress has been replicated with non-BC-PS sources of PS. Fahey & Pearl (1998) reported intake of 800 mg/d of S-PS for two weeks reduced post exercise cortisol responses to intensive resistance training by 20% in males. Similarly, Starks, Starks, Kingsley, Purpura, & Jaeger (2008) demonstrated 600 mg/d of S-PS for 10 days was sufficient to significantly attenuate plasma cortisol responses before and during exhaustive bicycle ergometer (85% VO_{2max}) exercise in young males. However, both of these exercise stress studies, and those of Monteleone et al. (1990, 1992), comprised relatively small study samples (N = ≤ 11).

Further studies have examined the potential for PS to moderate HPA axis-mediated stress responses following psychosocial stress induction. A dose-dependent effect of a soy lecithin phosphatidic acid and PS complex (PAS) has been reported. Hellhammer et al. (2004) administered 400, 600, 800 mg/d of PAS, or a placebo to a mixed sample for three weeks in an independent groups design. Following exposure to the TSST only participants supplemented with 400 mg of PAS demonstrated significant attenuation of plasma and salivary cortisol across the stress response profile. Salivary cortisol was particularly affected with a 20% reduction compared to placebo. A parallel reduction in ACTH was considered to suggest a potential central dampening effect on the HPA axis. A post-hoc factor analysis derived measure of distress, taken from the Spielberger's State Anxiety Scale, also showed attenuated perceived distress in 400 mg/d treated participants. No significant effects on endocrine parameters in the 600 and 800 mg/d conditions were observed.

1.5.4 Phospholipids, Stress, and Cognitive Performance

Evidence of the relationship between cortisol and impairment of cognitive performance and potential capacity of PL supplementation to attenuate HPA axis-mediated stress responses, has informed studies designed to examine the potential for PLs to protect cognitive performance under conditions of stress (for summary of studies see Table 1.2).

Parker et al. (2011) reported protective effects of S-PS on cognitive performance following intensive lower body resistance training. A two week intake of 400 mg/d S-PS improved accuracy and time needed for completion on a serial subtraction test compared to placebo in young males. Plasma cortisol and mood were unaffected by S-PS intake. Further studies have offered some support for a stress-buffering effect of PLs but failed to demonstrate significant protective effects on cognitive performance. For example, an EEG study reported the potential for a 200 mg/d six week intake of S-PS to induce a relaxed state in young men (Baumeister et al., 2008). Participants were stressed by exposure to a delayed auditory feedback task before completing the Stroop-colour word interference and D2 concentration tests. No significant effects of stress on cognitive performance were revealed. However, a significant pre- and post-stress decrease in right hemispheric frontal lobe Beta-1 power was reported. Heightened Beta-1 spectral power has been positively associated with cognitive task demand and visual information overload (Adey, 1997; Fernandez et al., 1995; Ray & Cole, 1985). Consequently, reduced Beta-1 spectral power in S-PS treated participants was interpreted as indicative of an increased relaxed state following S-PS intake. No measures of cortisol were collected in this study so effects on the HPA axis responses are unknown.

A number of studies suggests that the effects of PL supplementation may only be shown in individuals characterised by some form of increased ‘stress vulnerability’. Benton, Donohoe, Sillance, & Nabb (2001) reported that the administration of 300 mg/d of S-PS for 30 days reduced subjective stress responses and improved mood in a young mixed sex sample during a mental arithmetic stressor. This modulation of subjective responses was only demonstrated in participants scoring highly on a neuroticism scale. Furthermore, high neuroticism has been previously shown to moderate improved mood and blunt cortisol responsivity following administration of a bovine-derived whey protein (alpha-lactalbumin; Markus et al., 2000).

Hellhammer, Waladkhani, Hero, & Buss (2010) reported lower subjective stress and increased perceived control after TSST exposure in adult males (30 – 55 years) treated with a bovine-derived PL-enriched drink (providing 13.5 g of PL) for three weeks compared to a placebo. A trend for improved RT for an item recognition WM task was also reported (p = .09). This significance increased when individual variability in cortisol response was controlled (p = .06). In the whole sample no effect on endocrine responsivity was observed aside from a tendency towards lower percent maximum salivary cortisol increase in the PL condition. To explore the potential for stress vulnerability to moderate the potential stress buffering effect of PL intake, a median split of high and low stress participants based upon scores on the Trier Inventory of Chronic Stress (TICS) was undertaken. Distinct analyses of high stress participants across treatment conditions revealed a dampening of ACTH and cortisol in the PL treated condition.

A similar study which considered high scores on the TICS to recruit stress vulnerable older adults (30 – 51 years) at study entry administered two concentrations of a bovine PL enriched drink (150 mg/d & 300 mg/d PL) or a placebo for six weeks (Schubert, Contreras, Franz, & Hellhamrner, 2011). No significant effect on acute salivary cortisol responses following TSST exposure was reported. However, a post-hoc split of participants based upon age revealed that older participants (41-51 years) receiving the 300 mg/d PL drink demonstrated a significantly higher visual performance score on a test of visuospatial memory (VISGED; see Section 3.5.3.3 for description of this test) that was not evident pre-stress.

Schubert et al. (2011) also assessed salivary CAR pre- and post-PL/placebo intake. Participants supplemented with the 300 mg/d PL drink demonstrated a delayed decline in salivary cortisol following the CAR peak (demonstrated in the 150 mg/d PL condition by trend). The CAR has been proposed to be involved in the mobilisation of energy resources for the coming day (Pruessner, Wolf et al., 1997), shows variation dependent upon the anticipated demands of specific days (Fries, Dettenborn, & Kirschbaum, 2009), and may be attenuated in individuals under a high chronic stress-load (O'Connor et al., 2009). The authors interpreted the moderation of CAR by PL intake as potentially protective in individuals facing chronic stress via the attenuation of the chronic stress-induced decline in available cortisol after awakening expected in the high stress-load group.

Hellhammer, Hero, Franz, Contreras, & Schubert (2012) provided further evidence of stress-load as a potential vulnerability factor. Additionally, cortisol responder type (high or low) emerged as a potential modulating factor in the effect of PL intake. Stress responses to the TSST in men (aged 30 – 55 years) before and after a 12 week daily supplementation of an omega-3 PL-rich capsule (providing 300 mg/d of PS) or placebo were examined. No overall effect of treatment on salivary cortisol response to TSST was found. An effect of repeated TSST exposure and classification of high or low cortisol responder (post initial TSST) on cortisol response was reported. High responders exhibited higher response profiles than low responders across both TSST exposures. A potentially beneficial moderation of cortisol response to acute stress by omega-3 PL intake in cortisol responder groups was also observed. A trend for attenuated cortisol in high cortisol responders and increased cortisol in low cortisol responders was revealed. The authors suggest the effects of PL intake may be characterised by a normalisation of the cortisol response. Planned post-hoc analyses revealed high chronic stress participants (categorised by TICS score) supplemented with omega-3 PS reported lower perceived chronic stress.

Table 1.2 Summary of studies examining the effects of PL supplementation on stress or stress and cognition

Authors	Sample	Study Design	PL form	Dose	Intake Period	Stressor	Endocrine Response	Subjective Response	Cognitive Perfomance	Other Effects
Monteleone et al. 1990	8 males, 32.1 ± 1.7 years (*mean* ± *SEM*)	DB PC crossover	BC-PS	50 & 75 mg	Acute IV	Physical (bicycle ergometer)	Plasma CORT & ACTH ↓ (both doses)	NM	NM	No effect on SBP, DBP, HR
Monteleone et al. 1992	9 males, 29.2 ± 2.2 years (*mean* ± *SD*)	DB PC crossover	BC-PS	800 & 400 mg/d	10 days	Physical (bicycle ergometer)	Plasma CORT & ACTH ↓ (800 mg dose)	NM	NM	No effect on SBP, DBP, HR
Fahey & Pearl, 1998	11 males, 22.8 ± 3.4 years (*mean* ± *SD*)	DB PC crossover	S-PS	800 mg/d	2 weeks	Physical (Resistive exercise-induced overtraining)	Plasma CORT ↓	Subjecitve well-being ↑	NM	
Benton et al., 2001	48 males, 20.8 ± 2.6 years (*mean* ± *SD*)	RCT DB PC	S-PS	300 mg/d	30 days	Mental arithmatic task	NM	Subjecitve mood (post stress) ↑in high neuroticism participants	NM	No effect on SBP, DBP, HR
Hellhammer et al, 2004	79 mixed (10 males/females per condition) 24 - 45 years	DB PC	Soy lecithin phosphatidic acid & PS complex	400, 600, & 800 mg/d	3 weeks	TSST	Plasma & salivary CORT, & ACTH ↓ (400 mg dose)	Perceived distress ↓(400 mg dose)	NM	No effect on HR
Starks et al., 2008	10 males, 26.2 ± 1.5 years (*mean* ± *SEM*)	DB PC crossover	S-PS	600 mg/d	10 days	Physical (bicycle ergometer)	Plasma CORT ↓ Plasma testoterone to CORT ratio ↑	NM	NM	
Baumeister et al., 2008	16 males, 25 ± 3 years (*mean* ± *SEM*)	RCT DB PC	S-PS	200 mg/d	6 weeks	Delayed auditory feedback task	NM	NM	No effect on Stroop & D2 concentration tests	Reduced Beta-1 spectral power (EEG)
Hellhammer et al, 2010	45 males 30 - 55 years (*mean* = 41.6)	RCT DB PC	BM-PL rich drink	13.5 g/d (PL)	3 weeks	TSST	No overall effect on plasma or salivary CORT Trend for ↓ ACTH and cortisol in high stress load (TICS) participants (post hoc)	Perceived distress ↓ Perceived controllability ↑ (post hoc)	Trend of faster reaction time	
Parker et al., 2011	18 males 22.5 ± 2.2 years (mean ± SEM)	RC DB PC crossover	S-PS	400 mg/d	2 weeks	Physical (lower body resistance training)	No effect on plasma CORT	No effect on mood (POMS)	Serial subtraction task ↑	No effect on SBP, DBP, HR
Schubert et al., 2011	73 high stress load (TICS) males 30 - 51 years	RCT DB PC	BM-PL rich drink	150 mg/d & 300mg/d (PL)	6 weeks	TSST	No effect on acute salivary CORT Delayed decline from CAR peak in salivary CORT (300 mg/d)	NM	Visual memory performance ↑ in older participants (41-51 years; 300 mg/ dose)	
Hellhammer et al., 2012	60 males 30 - 55 years (*mean* placebo = 42.27 PL = 43.90)	RCT DB PC	Omega-3 PL-rich capsule	300 mg/d (PS)	12 weeks	TSST	No overall effect on plasma or salivary CORT Trend for moderation of CORT response by responder type (high ↓ low ↑)	Perceived chronic stress (TICS) ↓(high stress load participants)	NM	No effect on HR

BC-PS - bovine cortex phosphatidylserine. S-PS - soy-derived phosphatidylserine. BM-PL - bovine milk phospholipids. mg/d - milligrams per day. IV - intravenous. CORT - cortisol. TSST - Trier Social Stress Test. NM - not measured. POMS - Profile of Mood States. DB - double-blind. PC - placebo controlled. TICS - Trier Inventory for Chronic Stress. CAR - cortisol awakening response

1.5.5 Proposed Mechanisms of Phospholipid Action

Animal models have been employed to characterise potential protective mechanisms of PLs on cognitive performance in the aged brain. Performance improvements in rats have been proposed to be underpinned by the attenuation of physiological, neurochemical and morphological changes associated with the ageing process by PL (predominantly PS) supplementation (Pepeu, Pepeu, & Amaducci, 1996).

The mechanism underpinning the effect of PL supplementation on the activity of the HPA axis is poorly characterised. However, the observed effects of PLs on cell membranes are a potential candidate mechanism. Phosphatidylserine is involved in the regulation of cell membrane fluidity and therefore regulation of cell to cell communication (Vance & Steenbergen, 2005). By acting upon the lipid microviscosity of cell membranes, PS may affect receptor-ligand interactions via modification of the position of membrane proteins with enzymatic functions (Hirata & Axelrod, 1980; Monteleone et al., 1992). Via direct and indirect interactions with key cell membrane associated proteins (e.g., PKC), PS has been shown to modulate receptor, ion channel, enzyme, and signalling molecule activity (Canonico & Scapagnini, 1989; Pepeu et al., 1996). Rat models have also demonstrated the potential for PS administration to affect the number of receptors in cell membranes (Stockert, Buscaglia, & Derobertis, 1989). By altering receptor-ligand interactions and receptor density, PL supplementation has a hypothesised potential to affect receptor interactions that underpin the HPA axis activation cascade. Indeed, evidence of the capacity of PS supplementation to attenuate ACTH suggests a central dampening effect (Hellhammer et al., 2004). One hypothesised mechanism is that PS treatment may alter CRH receptor interactions which could attenuate activation of the HPA axis (Monteleone et al., 1992). However, there is no direct evidence to support such a mechanism.

It should be noted that the amount of PLs that reach the CNS after oral or intraperitoneal administration may be very small (Pepeu et al., 1996). For example, only 0.01% of PS was detected in the rat brain after acute intraperitoneal injection (Bruni et al., 1989). However, most effects of PL intake are reported after chronic administration. This suggests that the accumulation of PLs in the brain may be needed for observed effects. Some evidence of a continued effect of PS after cessation of chronic administration in animal models (Vannucchi & Pepeu, 1987) and aged adults with Alzheimer's disease (Amaducci, 1988) offers some support for this hypothesis.

1.5.6 Summary of the Effects of Phospholipids on Stress and Cognition

Early promising evidence of the stress-buffering effects of BC-PS on ACTH and cortisol after exercise induced stress has received some support from later physical and psychosocial stress studies employing non-bovine cortex PL sources. Effects of soy and bovine milk-derived PS, PL rich drinks, and PL combined with lecithin phosphatidic acid or omega-3, on endocrine and psychological parameters of stress have been reported. The majority of studies have focussed upon the supplementation of PS, administering between 200 and 800 mg/d doses for 10 days to 3 months.

Whilst a number of studies have reported the attenuation of endocrine and subjective stress responses in a general sample, the effects of PL supplementation are often shown in individuals characterised by some form of stress vulnerability. Stress-load, cortisol responder type (high or low responsivity), or an assumed tendency for greater stress reactivity associated with a personality trait (neuroticism), may moderate the potential for PL supplementation to dampen stress responses.

The capacity of PL supplementation to protect cognitive performance from the impairing effects of stress has received inconsistent support. Better performance after PS supplementation has been reported. Hence, a PL-rich drink resulted in marginally significant improvement in WM RT, and improved visual memory in a subgroup of participants. However, further studies have reported no effect of PL treatment. The relationship between PL intake and cognitive performance under stress may be further complicated by an inconsistency in the reported effects of stress on cognition. Methodological differences between studies (e.g., time of day), the magnitude of the stress response elicited, and variability in the sensitivity to stress of specific cognitive tests employed have been demonstrated to modulate the observed effects of stress on cognition. Such factors may explain some of the inconsistency in studies of PL supplementation.

A number of hypothesised mechanisms for observed effects of PL on stress responses have been proposed. However, it is evident that a clearly characterised mechanism of action has yet to be defined.

Chapter 2:
Thesis Aims and Objectives

Chapter 2 Thesis Aims and Objectives

2.1 General Aims

The aims and objectives of the thesis were two-fold. The thesis end-point was a planned intervention study to assess the potential for a bovine-derived PL intervention to modulate cognitive performance and stress responses under conditions of stress. Considering the small and inconsistent reported evidence of the potential for PLs to moderate cognitive performance under stress conditions, the examination of a number of design and methodological factors was considered an important prerequisite for this intervention study. The preparatory identification of suitable design and methodological conditions for the PL intervention study constitutes the second aim and objective of the thesis.

The thesis studies preceding the dietary intervention study were undertaken with the aim of identifying the appropriate methodological context in which to undertake an intervention study. These methodological considerations can be summarised as:

1. Identification of an appropriate stress context
2. Identification of specific cognitive tests sensitive to stress exposure
3. Identification of the characteristics of an appropriate sample

2.2 Stress Context

The identification of a reliable stress context for the PL intervention study is a central aim of the thesis. Considering the centrality of cortisol in both the moderation of cognitive performance, and potential stress-buffering effect of PLs, a stressor that reliably provokes the HPA axis was required. The majority of PL intervention studies to date have employed laboratory-based stress contexts. Laboratory protocols offer the benefit of standardisation of the stress faced, systematic and reliable measurement of stress responses, and control of extraneous variables. However, laboratory stress tasks typically employ artificial stimuli rarely encountered in the real-world and less intimately linked to genuine goals and identity. Furthermore, evidence of only a moderate relationship between cortisol responses elicited in the laboratory and real-world contexts have been reported (Van Eck et al., 1996). Hence, the feasibility of employing a real-world or a laboratory stress contexts was explored and reported in Chapters 4 and 5.

A mixed design comprising a between-subjects random allocation to a PL-drink or placebo condition, and a within-subjects repeated exposure to stress following PL or placebo drink intake was adopted for the PL intervention study (reported in Chapter 7). The increased power and reduction of random effect variation were the main considerations underpinning the decision to examine cognitive performance under stress in the same participants pre- and post-dietary intervention. This was considered important due to the modest to small effects reported for PL intervention studies, combined with the high inter-individual variability in cortisol response and cognitive test performance. The benefits offered by this design create a number of key methodological considerations. Firstly, as habituation is an often reported characteristic of the HPA axis, repeated exposure to a homotypic stressor will likely result in non-comparable endocrine responses across repeated stress exposures. Comparing stress responses and cognitive performance before and after a nutritional intervention without comparable cortisol responsivity across the repeated exposures increases the likelihood that any effect of the nutritional manipulation will be confounded, or overshadowed, by the divergent background endocrine milieu. Therefore the capacity of stress contexts to provoke significant cortisol responses over repeated exposures was explored.

2.3 Cognitive Tests Sensitive to Stress

Previous evidence of the limited capacity of PL intake to moderate cognitive performance under conditions of stress may be partly reflective of the divergent sensitivities of specific cognitive tests to stress provocation. Examination of the effects of stress on cognitive performance, and the potential for a PL dietary intervention to modulate this relationship, requires the use of suitable cognitive tests shown to be sensitive to stress conditions. Specific tests of cognitive domains sensitive to stress exposure were administered under stress and non-stress conditions across the studies in this thesis. This permitted the selection of tests, showing capacity for impaired performance under stress conditions, to explore the potential protective effects of PL intake.

2.4 Selection of Sample

The stress and cognitive performance literature suggests that the modulating effects of stress on cognition may only be demonstrated in those exhibiting robust cortisol responses. Furthermore, evidence of the potential for PL interventions to moderate

cortisol responsivity, and by extension protect cognitive function, suggests such effects may be limited to individuals with some form of stress vulnerability. Hence, individuals with a tendency towards high cortisol responses to stress provocation were targeted for the PL intervention study. The existence of stable dispositional factors related to cortisol responsivity was explored via the collection of trait personality measures in studies utilising real-world (Chapters 4 and 5) and laboratory contexts (Chapter 6). Emergent predictors of cortisol responsivity were then employed to select a sample for the PL intervention study (Chapter 7).

2.5 Specific Thesis Aims

I. Explore the utility of a real-world or laboratory stressor for a dietary intervention study (Chapters 4 – 6)

II. Assess the capability of the identified stress context to elicit comparable stress (primarily cortisol) responses over repeated exposures (Chapter 6)

III. Identify cognitive tests sensitive to the impairing effects of stress (Chapter 5 and 6)

IV. Identify potential predictors of cortisol responsivity to aid selection of an intervention sample likely to elicit robust cortisol responses (Chapters 4 – 6)

V. Examine the effects of a dietary PL intervention on cognitive performance and stress responses under conditions of stress (Chapter 7)

Chapter 3:
General Methodologies

Chapter 3 General Methodologies

3.1 Introduction

A number of methods, protocols, and psychometric and cognitive performance measures are common to multiple studies included in this thesis. The general methodologies for the use of these measures are detailed here. The administration of, and any specific adjustments to, these measures are detailed in the methods section of each respective experimental chapter.

3.2 Measurement of Stress Response

A wide range of psychological and physiological indices of stress responsivity have been employed in the study of stress. The following methods were utilised across the studies included in this thesis to assess endocrinological, cardiovascular and subjective responses to stress.

3.2.1 Measurement of Salivary Cortisol

Salivary cortisol level was used as a proxy measure of adrenal, pituitary and hypothalamic function. Due to its liposoluble nature, unbound cortisol can easily enter nucleated cells via passive diffusion. Unbound cortisol appears in all bodily fluids including blood, cerebral spinal fluid, sweat, urine, semen, and saliva. Unbound cortisol enters saliva primarily via the acinar cells of the parotid, submandibular and sublingual salivary glands. However, the concentration of cortisol in saliva is independent of salivary flow rate (Gatti et al., 2009). A strong positive correlation between levels of unbound cortisol in saliva and plasma (approximately 80% of total variance [$r \geq .90$]) has been reported (Arafah, 2006; Gozansky, Lynn, Laudenslager, & Kohrt, 2005; Kirschbaum & Hellhammer, 1989; Vining, McGinley, Maksvytis, & Ho, 1983). Salivary analysis is therefore considered a physiologically relevant and useful measure of the unbound fraction of cortisol. Salivary cortisol is highly correlated with total cortisol levels in blood (Kirschbaum & Hellhammer, 1989, 1994). However, absolute cortisol levels in saliva are lower due to the metabolising action of enzyme 11β-hydroxysteroid dehydrogenase type 2 (Van Uum et al., 2002).

The use of saliva to measure cortisol was considered to confer a number of advantages over alternative sampling methods. Collecting blood samples is invasive and associated

with stress-induced adrenal activation that would likely confound endocrine responses to stress protocols (Granger et al., 2007). Measurement of cortisol in urine is a useful method for measuring 24 hr aggregated basal activity but is not suitable for assessing rapid changes in cortisol response. Saliva for assay can be easily collected repeatedly over relatively short time intervals using non-invasive sampling methods.

All saliva samples were collected using a Salivette® saliva collection device - a widely utilised and validated method (Arafah, Nishiyama, Tlaygeh, & Hejal, 2007; Dorn, Lucke, Loucks, & Berga, 2007; Gatti et al., 2009; Gozansky et al., 2005; Hellhammer, Wust, & Kudielka, 2009). Cortisol assayed from Salivettes® has also been shown to be a better predictor of bound and unbound plasma cortisol compared to passive drool methods (Poll et al., 2007). In the studies presented in this thesis, samples were collected via the chewing of a roll-shaped synthetic saliva collector swab stored in a sample tube. Participants were asked to place the saliva collector swab directly into their mouths from the Salivette® tube and chew the swab gently for at least one minute to ensure adequate saliva absorption. The swab was then returned directly from the mouth into the Salivette® tube. Saliva collection was performed at least 1 hour after consumption of meals and drinking caffeine/acidic drinks or brushing teeth to ensure no contamination of the saliva by interfering substances (see Appendix 1 for cortisol collection standard operating procedures). The number and frequency of samples collected from participants was informed by prior research demonstrating typical acute cortisol responsivity to stress. Cortisol concentrations following acute stress peak 21 – 40 minutes after stressor onset (Dickerson & Kemeny, 2004) with a gradual return to baseline levels after approximately one hour (Kirschbaum & Foley, 2010). All studies outlined in the thesis collected at least one baseline measure prior to stress exposure and further samples at approximately 10 minutes intervals until approximately 40 – 50 minutes after stress onset to adequately capture the cortisol response trajectory.

Due to the diurnal variation in cortisol secretion, time of day is an important methodological consideration when sampling this steroid. Firstly, testing early in the morning can interfere with, and be confounded by, the CAR. Secondly, HPA diurnal activity follows a pronounced circadian rhythm characterised by an early morning peak of secretory bursts of cortisol, and decreasing bursts over the afternoon. Consequently, basal cortisol levels vary as a function of time of day. The pattern and net increase in cortisol response to acute psychosocial stress has been shown to be comparable when testing occurs between the hours of 0945 – 1900 hrs (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004). However, higher baseline pre-stress salivary cortisol

levels in the morning contribute to significantly higher morning area under the curve (AUC). Higher baseline cortisol levels have also been negatively associated with lower net increases in acute stress suggesting higher baseline levels may provide less “space” for an effect of stress (Decherney et al., 1985; Hermus, Pieters, Smals, Benraad, & Kloppenborg, 1984; Kudielka, Schommer et al., 2004; Schurmeyer et al., 1987). Moreover, Dickerson and Kemeny’s (2004) meta-analytic review reported that time of day significantly predicted cortisol response effect sizes (afternoon, *d* = 0.46; morning, *d* = 0.14).

Considering the potential for the influence of divergent baseline endogenous levels to affect measures of cortisol response, testing in the studies presented in the thesis was confined to the afternoon. All salivary cortisol measures across the reported studies were collected after 1200 hrs (with the exception of four participants tested at 1145 hrs in Study 1 due to constraints of the naturalistic testing context) and before 1720 hrs. In studies requiring participants to attend repeated experimental visits, the repeat visiting times were matched to within 1 hr of the first visit.

3.2.1.1 Assay of salivary cortisol

Saliva was extracted from cotton wool swabs by centrifugation (2500 rpm, five minutes) and frozen at - 20°C until assay. Salivary-free cortisol concentrations were determined using a commercially available Salimetrics Salivary Cortisol Enzyme Immunoassay kit (EIA; Sarstedt; Nümbrecht, Germany). The Salimetrics EIA kit is a competitive immunoassay with a high sensitivity (< 0.007 ug/dL) specifically designed and validated for the quantitative measurement of salivary cortisol. The assay uses a microtitre plate coated with monoclonal antibodies to cortisol. Cortisol in standards and unknowns compete with cortisol linked to horseradish peroxidase for antibody binding sites. Once unbound components are washed away, the reaction of peroxidise enzyme on the substrate tetramethylbenzidine (which produces a blue colour) is used to measure bound cortisol peroxidase. Sulphuric acid is used to stop the reaction, forming a yellow colour. The level of cortisol peroxidase is indicated by the intensity of colour which is inversely proportional to cortisol level (Chard, 1990).

Intra- and inter-assay coefficients of variability were below 9.5 and 12.5% respectively across all studies (below the respective < 10 and < 15% levels recommended by the assay kit manufacturer). Specific coefficients of variability values are reported in the method sections of each respective study.

3.2.1.2 Aggregated measures of cortisol

A number of aggregated indices of cortisol response were included in the statistical analyses across all studies. The delta increase in cortisol response was calculated by subtracting the baseline cortisol value from the peak post-stress induction level. Area under the curve formulae were used to calculate aggregated measures of cortisol response. Two formulae for calculating the area under the curve using the trapezoid method were employed (Pruessner et al., 2003): area under the curve with respect to ground (AUCg), and area under the curve with respect to increase (AUCi). In relation to endocrinological data AUCg is considered to give an indication of total hormonal output independent of changes over time, and AUCi indexes response change over time (Pruessner et al., 2003). The trapezoid method uses the measurement and time distance between measurements to calculate AUC values. Two formulae for each aggregated AUC measure were used dependent upon whether the time differences between measurement sampling points were equal or unequal. The following formulae were employed:

$$AUCg = \sum_{i=1}^{n-1} \frac{m_{(i+1)} + m_i}{2}$$

m_i = each individual measurement
t_i = individual time between each measurement
n = total amount of measurements

Equation 1.1 Formula for the calculation of AUCg with equal time differences between sampling time points

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

m_1 = first measurement

Equation 1.2 Formula for the calculation of AUCi with equal time differences between sampling time points

$$AUCg = \sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i) * t_i}{2}$$

Equation 1.3 Formula for the calculation of AUCg with unequal time differences between sampling time points

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

Equation 1.4 Formula for the calculation of AUCi with unequal time differences between sampling time points

3.2.2 Cardiovascular Measures

Measures of cardiovascular response were collected as proxy measures of adrenergic reactivity to stress exposure. The specific dynamics of cardiovascular response to stress shows some variability dependent upon the nature of the stressor and individual differences (Durel et al., 1993; Herd, 1991). However, exposure to acute stress typically activates the sympathetic and inhibits the parasympathetic nervous systems. Sympathetic activation and parasympathetic withdrawal increases heart rate (HR). Cardiac output is increased via increased HR and venoconstriction mediated increased

stroke volume. Blood pressure is elevated by increased cardiac output and a net increase in systemic vascular resistance mediated by vasoconstriction (renal and splanchnic) and vasodilatation (skeletal muscle and adipose tissue). A pulse pressure waveform transmits through the arterial tree after each left ventricle contraction of the heart emits a bolus of blood into circulation. Peak pressure in the arterial system is reached during this cardiac contraction phase (systole). As the heart relaxes (diastole) pressure in the arterial system drops just prior to onset of next systolic phase (Sherwood & Cartels, 2010). Measures of systolic (SBP) and diastolic (DBP) are the most commonly employed measures of arterial blood pressure. Blood pressure is measured in millimetres of mercury (mmHg). The studies presented in this thesis employed measures of SBP and DBP across all studies. Heart rate was also measured in the laboratory-based studies.

Ensuring adequate baseline conditions for the measurement of resting cardiovascular parameters is crucial as meaningful assessment of cardiovascular reactivity to stress is largely dependent upon the basal measure used for comparison. Consequently, cardiovascular measures at baseline temporally removed from stress onset, were collected. For the naturalistic studies in the thesis, cardiovascular measures were collected a minimum of 20 minutes pre-stress onset. Laboratory-based studies in this thesis afforded more control over baseline measure collection. A one hour resting period was completed prior to the collection of cardiovascular baseline measures. Individuals presenting with a resting blood pressure ≥ 140/90 mmHg over repeated measurements at screening were excluded from further participation. Increases in blood pressure are expected as a result of exposure to acute stress. However, increases in SBP in excess of 180-185 mmHg resulted in the termination of testing to ensure the safety of participants.

Two validated ambulatory blood pressure monitors were employed to collect measures of cardiovascular response at the brachial artery of the upper (non-dominant) arm. The Omron M7 (HEM-780-E; Omron Healthcare, UK) is an automated oscillometric upper arm BP monitor validated for use in healthy and clinical cases (Coleman et al., 2008; El Feghali et al., 2007). The monitor measures SBP and DBP. This monitor was used in naturalistic studies as participants were not willing to wear an ambulatory BP monitor during audition exposure. The monitor was fitted before and after audition exposure but removed during the audition. For laboratory based studies, a Spacelabs 90207 (Spacelabs Medical Inc., USA) automated oscillometric upper arm ambulatory BP monitor was employed. This widely validated monitor (Amoore & Geake, 1997; Marquez

Contreras et al., 1998; O'Brien, Mee, Atkins, & O'Malley, 1991) measures SBP, DBP and HR. The Spacelabs 90207 is designed to be worn for long periods of time and can be worn by ambulatory participants. This monitor was worn throughout experimental visits. Cardiovascular measures were all taken whilst participants were seated in every study.

3.2.3 Subjective Measures

3.2.3.1 The Stress and Arousal Checklist (SACL)

The SACL (Mackay, Cox, Burrows, & Lazzerini, 1978) is a 30-item adjective list of self-reported feelings of stress (18 items) and arousal (12 items; Appendix 2). The two-dimensional SACL model of stress comprises feelings of pleasantness and unpleasantness or hedonic tone (stress) related to subjective response to the environment, and vigorousness (arousal) that is considered to reflect perceived autonomic arousal (King, Burrows, & Stanley, 1983). Respondents are required to indicate the extent to which each adjective (e.g., stimulated, apprehensive, up tight) describes how they are feeling at this moment in time. Responses are made with reference to a four-point Likert scale: *definitely describes your feelings (++), more or less describes your feelings (+), cannot decide whether it describes how you feel (?),* and *does not describe the way you feel (-*). The long scoring method (four-point Likert scale) was employed (++ = 4; + = 3; ? = 2; - = 1; scores are reversed for negatively weighted items; Mackay et al., 1978). The score ranges for the long scored version of the SACL are: stress, 18 – 72; arousal, 12 – 48. Alternative ordered versions of the SACL were administered at each time point to avoid habituation in response. The SACL has been used extensively as a measure of momentary subjective response to stress induction. Several validation studies have been published (e.g., Fischer & Donatelli, 1987; King et al., 1983). Reliability coefficients (Cronbach's alpha; r_α) for each scale have been reported: stress, r_α = .86 – .89; arousal, r_α = .74 – .84 (King et al., 1983; Mackay et al., 1978).

3.2.3.2 The Profile of Mood States (POMS)

The POMS (McNair, 1971) is a self-report adjective checklist of 65 items measuring distinct transient affective mood states (Appendix 3). The scale comprises six mood subscales (the score range for each dimension is given in parentheses): *Tension-anxiety* (0—36), *Depression-dejection* (0—60), *Anger-hostility* (0—48), *Vigour-activity*

(0—32), *Fatigue-inertia* (0—28) and *Confusion-bewilderment* (0—28). A *Total Mood Disturbance* (TMD; 0 – 200) score can be calculated by summing all the negative valenced subscales (all subscales except Vigor-activity). Respondents are required to indicate the extent to which each adjective (e.g., lively, restless, uneasy, helpless) describes how they are feeling at this moment in time. Responses are made in reference to a five-point Likert scale: *not at all* = 0*, a little* = 1*, moderately* = 2*, quite a bit* = 3*,* and *extremely* = 4. Likert scoring is reversed for negatively weighted items. Reliability coefficients for the 65-item POMS scale range between r_α = .63 – .92 for subscales, and r_α = .75 – .92 for total score (Norcross, Guadagnoli, & Prochaska, 1984; Nyenhuis, Yamamoto, Luchetta, Terrien, & Parmentier, 1999).

3.2.3.3 The Profile of Mood States – Short form (POMS-SF)

The POMS-SF (Shacham, 1983) is a shortened version of the original POMS comprising a self-report adjective checklist of 37 rather than 65 items (Appendix 4). The scale still comprises six mood subscales (the adjusted score range for each dimension is given in parentheses): *Tension-anxiety* (0—24), *Depression-dejection* (0—32), *Anger-hostility* (0—28), *Vigour-activity* (0—24), *Fatigue-inertia* (0—20), *Confusion-bewilderment* (0—20) and *TMD* score (0 – 124). Correlation coefficients between subscale scores and TMD for POMS-SF and the standard POMS all exceed *r* = .95 (Shacham, 1983).

3.2.3.4 Primary Appraisal Secondary Appraisal (PASA)

The PASA (Gaab et al., 2004) is a measure of perceived threat and coping appraisal (Appendix 5). The PASA was originally developed to assess cognitive appraisal processes related to exposure to the TSST. The PASA comprises two situation-specific subscales that assess primary appraisal of the anticipated stressor (reliability coefficients for each subscale are shown in parentheses): *Challenge* (r_α = .64) and *Perceived Threat* (r_α = .85); and two subscales that assess secondary appraisal of coping ability: *Self-concept of One's Own Competence* (r_α = .79) and *Control Expectancy* (r_α = .76). Primary and secondary subscales can be combined into two summary scales: *primary appraisal* (r_α = .80) and *secondary appraisal* (r_α = .74). Respondents are required to indicate the extent to which they agree with 16 statements relating to the perception of the anticipated stressor. Statements relate to perception of the anticipated event as stressful, challenging, controllable or irrelevant (primary appraisal; e.g., "I do not feel threatened by the situation"), and assessment of available

coping resources (secondary appraisal; e.g., “I can think of lots of solutions for solving this task”). Responses are made with reference to a six-point Likert scale: *totally disagree* = 1*, rather disagree* = 2, *disagree to some extent* = 3*, agree to some extent* = 4*, rather agree* = 5*,* and *totally agree* = 6*.* Likert scoring is reversed for negatively weighted items.

3.2.3.5 Perceived Stress Reactivity Scale (PSRS)

The PSRS (Schlotz et al., 2011) is a measure of perceived stress reactivity (Appendix 6). The 23-item measure assesses general perceived reactivity to a number of stressful scenarios (e.g., social conflicts, high workload, and negative social evaluation). Respondents are required to choose from three Likert scale response options specific to each stressful scenario (e.g., “When I have many tasks and duties to fulfil..... *In general I stay calm, I usually get impatient,* or *I often get irritable”*). The PSRS has six subscales: *Reactivity to Work Overload, Reactivity to Social Conflicts, Reactivity to Social Stress, Reactivity to Failure, Anticipatory Reactivity,* and *Prolonged Reactivity*. An overall stress reactivity score can be computed by summing the subscales. High scores indicate higher stress reactivity. Reliability coefficients for the PSRS subscales range between r_{α} = .71 - .91, and retest reliability coefficients (r_{tt}) over a period of 7 months were r_{tt} = .63 - .84 (Schlotz et al., 2011; Schulz, Jansen, & Schlotz, 2005).

3.3 Screening Measures

A prominent feature of activation of the HPA axis by stress is the inter- and intra-individual variability in cortisol response. Research has demonstrated that cortisol responsivity to stress is influenced by numerous moderating and intervening factors (Biondi & Picardi, 1999; Dickerson & Kemeny, 2004; Kudielka, Hellhammer, & Wust, 2009). Awareness of modulatory factors can be used to inform the design of psychobiological research in which cortisol response is a primary outcome variable and potentially improve the signal to noise ratio. The recognition of potential confounding modulators influencing primary outcome variables is important considering the relatively modest effect sizes demonstrated in psychobiological research, and the tendency for small effect sizes in dietary intervention studies. Potential modulators of cortisol responsivity were assessed at screening and used to inform inclusion/exclusion criteria and selection of relevant covariates to include in statistical models used in the analysis of data presented in this thesis. Specific inclusion/exclusion criteria employed in each

study are detailed in each experimental chapter. Measures employed in the screening of participants are outlined here.

3.3.1 The Perceived Stress Scale (PSS)

The PSS (Cohen, Kamarck, & Mermelstein, 1983) is a 10-item self-report scale used to measure appraisal of perceived stress (Appendix 7). This global measure assesses how frequently respondents have experienced an uncontrollable, unpredictable or overloading situation during the last month, and the perceived effectiveness of individual ability and confidence to cope with this stress (e.g., "In the last month, how often have you felt that you were unable to control the important things in your life?"). Responses are made in reference to a five-point Likert scale: *never* = 0*, almost never* = 1*, sometimes* = 2*, fairly often* = 3*,* and *very often* = 4*.* Likert scoring is reversed for negatively weighted items. The PSS has been translated and validated widely in healthy and clinical populations. Reliability coefficients for the PSS range between r_{α} = .83 – .86 (Cohen et al., 1983).

The PSS was employed as a measure of perceived chronic stress level. Chronic stress can be defined as the prolonged and/or repeated exposure to a stressor or stressors. Animal (Akana et al., 1992; Brodish & Odio, 1989; Pecoraro, Reyes, Gomez, Bhargava, & Dallman, 2004) and human data (Chrousos & Gold, 1992; McEwen & Stellar, 1993) have demonstrated that chronic stress can modulate HPA axis function. There remains some heterogeneity in the literature regarding the effect of chronic stress upon acute cortisol response to challenge with evidence of no effect, hypo-, and hyper-reactivity reported (Kudielka, Hellhammer, & Wust, 2009; Kudielka, von Kanel, Preckel, Zgraggen, Mischler, Fischer et al., 2006; Melamed et al., 2006). The level or stage of chronic stress appears to be a key determinant of the relationship. An initial hyper-reactivity of the HPA axis to acute stress may be demonstrated in the early stages of exposure to chronic stress. Hyporeactivity may emerge later once a stage of exhaustion or burnout is reached (Kudielka, Bellingrath et al., 2006; Kudielka, von Kanel, Preckel, Zgraggen, Mischler, Fischer et al., 2006).

Considering evidence of an association with blunted cortisol responsivity, chronic stress was deemed an important factor to account for in sample selection. To reduce the potential influence of chronic stress upon study outcomes participants reporting high levels of perceived chronic stress (defined as ≥ 30 [PSS score range 0 – 40]) were

excluded. The PSS score of all study participants was included as a covariate in relevant statistical models.

3.3.2 The Hospital Anxiety and Depression Scale (HADS)

The HADS (Zigmond & Snaith, 1983) is a self-report measure widely used to assess the symptom severity and caseness of anxiety and depression in clinical and general populations (Appendix 8). The 14-item measure is comprised of two, 7-item, subscales: anxiety (HADS-A) and depression (HADS-D). Respondents are required to choose from four-point Likert scale response options specific to each item relating to how they have been feeling over the last week (e.g., “I feel tense of wound up.... most of the time, a lot of the time, from time to time/occasionally, and not at all”). Each item is scored 0 – 3 resulting in scores ranging from 0 – 21 for each subscale. Reliability coefficients for the anxiety subscale range between r_{α} = .68 - .93, and depression r_{α} = .67 - .90 (Bjelland, Dahl, Haug, & Neckelmann, 2002; Olsson, Mykletun, & Dahl, 2005). A number of different cut-off points have been used in the identification of “caseness” of anxiety or depression. Scores on either scale between 0 – 7 have often been adopted to represent 'no case', 8 – 10 indicate a 'possible case', and 11 – 21 suggestive of a 'probable case' of anxiety or depression. A review of the literature by Bjelland et al. (2002) revealed an optimal balance between sensitivity and specificity was achieved when a score of ≥ 8 was adopted as a cut-off point for caseness on both scales.

The modulation of cortisol response to acute psychosocial stress by affective mood disorders (e.g., major depression, anxiety disorder, and social phobia) has been widely reported. The difference between clinical and non-clinical populations appears to be particularly prominent when the HPA axis is challenged (Burke et al., 2005; Jessop & Turner-Cobb, 2008; Tsigos & Chrousos, 1994, 2002). Accordingly, the HADS was employed as a screening tool to exclude individuals with suspected affective mood disorders from participation. A cut-off point of ≥ 8 on either HADS subscale was adopted as an exclusion criterion across all studies presented in this thesis as indicative of potential mood disturbance (Bjelland et al., 2002). Respondents scoring above these cut off points were advised to seek further advice from their GP if they had any concerns about their mental health. Contact details of sources of mental health support were also provided in all participant information sheets in accordance with ethical principles.

3.3.3 Body Mass Index (BMI)

Body mass index is a commonly employed index of human body weight using mass and height to calculate underweight, overweight, and obesity cut off points. Body mass index is calculated by dividing body mass (weight in kg) by the square of height (in m) expressed in units of kg/m^2. The World Health Organisation currently classifies a BMI of $< 18.5\ kg/m^2$ as underweight, $\geq 25\ kg/m^2$ as overweight, and $\geq 30\ kg/m^2$ as obese (WHO, 2013).

The abdominal obesity phenotype has been associated with a number of alterations in HPA activity in both sexes. This includes altered ACTH secretion, increased reactivity to laboratory stressors, and hyper-responsiveness of the HPA axis to CRH and AVP (Pasquali et al., 2002; Pasquali et al., 1996). Emerging evidence also highlights a potential for increased impairment of cognitive performance under conditions of acute stress in individuals with abdominal obesity (Lasikiewicz, Hendrickx, Talbot, & Dye, 2013).

Considering the potential moderating effect of obesity on parameters of cortisol response individuals with a measured BMI $\geq 30\ kg/m^2$ were excluded from participation across all studies. The BMI of all eligible participants was included as a covariate in relevant statistical models.

3.3.4 Study Exclusion Criteria

The following exclusion criteria were common across studies (criteria specific to studies are stated in the method sections of respective study chapters):

- BMI $\geq 30\ kg/m^2$
- Medication use (prescribed and 'over-the-counter')
- Smoking
- Recreational drug use (last month)
- Current psychological affective/mood disorders (HADS subscale score > 8; Zigmond & Snaith, 1983)
- Perceived Stress Score ≥ 30 (Cohen et al., 1983)
- Endocrine, cardiovascular, or other chronic diseases (ascertained by a health screening questionnaire; Appendix 9)

- Hypertension. Participants with a resting BP exceeding 140/90 mmHg over repeated measurements at screening
- Pregnancy, planning pregnancy or lactating
- Night shift work

3.4 Potential Trait Predictors of Cortisol Responsivity

The identification of potential predictors of high cortisol responsivity was a primary objective of the thesis. Accordingly, a number of psychometric personality trait measures were collected across the studies presented in this thesis. Measures previously associated, or considered to have potential to be associated, with cortisol response to acute stress were employed to explore for potential trait predictors of cortisol responsivity (discussed in Section 1.3.2.1). The measures employed are outlined here.

3.4.1 Frost Multidimensional Perfectionism Scale (FMPS)

The FMPS (Frost et al., 1990) is a 35-item questionnaire that assesses multiple aspects of perfectionism (Appendix 10). The FMPS was one of the first scales to assume multiple dimensions of perfectionism. The FMPS is comprised of six subscales that embody this multidimensional perspective: *Concern over Mistakes* (9 items), *Personal Standards* (7 items), *Parental Expectations* (5 items), *Parental Criticism* (4 items), *Doubts about Actions* (4 items) and *Organisation* (6 items). A total perfectionism score can be calculated by summing all the subscales excluding Organisation[1]. Respondents are required to decide the extent to which a scale item describes them (e.g., "I am a neat person"). Responses are made with reference to a five-point Likert scale: *strongly disagree* = 1*, disagree* = 2*, neither agree nor disagree* = 3*, agree* = 4*,* and *strongly agree* = 5*.* Reliability coefficients for the subscales have been reported: CM, r_α = .86 – .88; PS, r_α = .82 – .83; PE, r_α = .84; PC, r_α = .82 – .84; D, r_α = .66 – .77; O, r_α = .89 – .93; and total perfectionism, r_α = .90 – .93 (Frost et al., 1990; Harvey, Pallant, & Harvey, 2004; Parker & Adkins, 1995; Purdon, Antony, & Swinson, 1999). Support for the construct validity of the FMPS comes from correlations with other perfectionism measures, namely: the perfectionism subscale from the Eating Disorder Scale *r* = .59

[1] Perfectionism: Organisation is omitted as this dimension has been shown to be poorly correlated with the other dimensions of the FMPS which are considered more reflective of the core nature of perfectionism

(Garner, Olmstead, & Polivy, 1983), and the Burns Perfectionism Scale r = .85 (Harvey et al., 2004).

3.4.2 Rotter's Internal-External Locus of Control Scale (LOC)

An abbreviated 13-item version of Rotter's (1996) LOC scale (Gurin, Gurin, & Morrison, 1978) was employed as a measure of perceived control orientation (Appendix 11). Developed by Rotter (1966), the construct focuses upon perceived causation of personal events and outcomes. The abbreviated LOC version employed here was a 13-item forced choice measure. For each item respondents are required to indicate which statement most accurately describes their opinion. Forced choice options are internally or externally orientated (e.g., "What happens to me is my own doing" and "Sometimes I feel that I don't have enough control over the direction my life is taking"). Responses are scored 0 for external orientation and 1 for internal orientation. Higher scores are indicative of high internal control orientation. Reliability coefficients of this LOC measure have been reported, e.g., r_{α} = .69 (Greenberger, Strasser, Cummings, & Dunham, 1989; Howell & Avolio, 1993).

3.4.3 Rosenberg Self-Esteem Scale (RSES)

The RSES (Rosenberg, 1965) is a 10-item self-report scale that remains one of the most widely utilised measures of global self-esteem (Blascovich & Tomaka, 1991; Appendix 12). Respondents are required to indicate the extent to which each scale item describes how they feel about themselves (e.g., "On the whole I am satisfied with myself"). Responses are made in relation to a four-point Likert scale: *strongly disagree* = 0*, disagree* = 1*, agree* = 2*,* and *strongly agree* = 3*.* Self-esteem scores range between 0 – 30. The Likert scale scoring is reversed for negatively weighted items. The RSES is a single-factor scale with scores ranging on a continuum from low to high self-esteem. Reliability coefficients typically range between r_{α} = .77 - .88; r_{tt} = .82 - .88 (Blascovich & Tomaka, 1991; Rosenberg, 1986).

3.4.4 Neuroticism

A 10-item neuroticism scale taken from the International Personality Item Pool (IPIP-N; Goldberg, 1999) was employed (Appendix 13). Neuroticism is a broad personality factor characterised by a tendency towards anxiety, depression, hostility, impulsiveness and self-consciousness. Neuroticism is considered a stable trait pervasive across context and situation. Respondents are required to indicate how accurately each scale item

describes how they feel about themselves (e.g., “I seldom feel blue”). Responses are made in reference to a five-point Likert scale: *very inaccurate* = 1*, moderately inaccurate* = 2*, neither accurate nor inaccurate* = 3*, moderately accurate* = 4*,* and *very accurate* = 5*.* The Likert scale scoring is reversed for negatively weighted items and high scores indicate higher neuroticism. The IPIP neuroticism scale shows high convergent validity with other measures that assess the ‘Five Factor’ broad personality traits (e.g., r = .73 for NEO PI-R; Goldberg, 1999).

3.4.5 Spielberger’s State Trait Anxiety Inventory (STAI)

The STAI (Spielberger, 1983) is a 40 item self-report measure that assesses two types of anxiety: state anxiety (20 items), and trait anxiety (20 items; Appendix 15). State anxiety refers to current perceived anxiety at the time of completing the measure. State anxiety assessment focuses upon perceived momentary autonomic arousal, tension, nervousness and worry (e.g., “I feel at ease”). Trait anxiety refers to the stable propensity to be anxious. Assessment of trait anxiety focuses upon perceived tendency for calmness, confidence and security (e.g., “I am a steady person”). Responses are made in reference to a four-point Likert scale specific to each anxiety subscale. For state anxiety respondents indicate the extent to which each scale item describes their feelings at the present moment: *not at all* = 1*, somewhat* = 2*, moderately* = 3*,* and *very much so* = 4*.* Trait anxiety responses are made in relation to the frequency of feelings of anxiety in general: *almost never* = 1*, sometimes* = 2*, often* = 3*,* and *almost always* = 4*.* Likert scoring is reversed for negatively weighted items.

Only the trait subscale of the STAI was administered in the studies described in this thesis. Reliability coefficients for the trait subscale range between r_{α} = .86 - .95, with test-retest reliability coefficients r_{tt} = .65 - .89 (Spielberger, 1983; Spielberger, 1989).

3.4.6 Cook-Medley Hostility Scale (Ho Scale)

The Ho Scale (Cook & Medley, 1954) is a 50-item scale derived from the Minnesota Multiphasic Personality Inventory that assesses dispositional hostility (Appendix 16). Respondents are required to indicate if each scale item is true or false (e.g., “It is safer to trust nobody” and “I think most people would lie to get ahead”). Responses are scored 0 or 1 depending upon the positive or negative weighting of each item. Higher scores indicate high hostility. Reliability coefficients for the Ho have been reported as r_{α} = .86

(Cook & Medley, 1954), retest reliability r_{tt} = .85 (one year; Barefoot, Dahlstrom, & Williams, 1983), r_{tt} = .84 (four years; Shekelle, Gale, Ostfeld, & Paul, 1983)

3.5 Measurement of Cognitive Performance

Two primary thesis objectives were 1) the identification of tests of specific cognitive domains sensitive to the impairing effects of stress, and 2) exploring the potential for a nutrient intervention to moderate this performance impairment under repeated acute stress conditions. The identification of appropriate cognitive tests was achieved by the selection of candidate cognitive domains, and specific tests, representative of those domains, evidenced as sensitive to impairment by previous stress research. Selected tests were subsequently piloted across early studies (Chapters 5 and 6) to ascertain suitability for use in the subsequent nutrient intervention study (Chapter 7).

An important consideration when assessing cognitive performance in repeated measures designs is the influence of practice effects (Bartels, Wegrzyn, Wiedl, Ackermann, & Ehrenreich, 2010; Basso, Bornstein, & Lang, 1999; Beglinger et al., 2005; Feinstein, Brown, & Ron, 1994). Repeat administration of the same test is likely to result in performance enhancement that could confound any effect of the independent variable(s) on performance. Methods of reducing the influence of practice effects on performance, adopted in all studies presented in this thesis, are detailed below.

3.5.1 Methods of Controlling for Cognitive Test Practice Effects

Serial cognitive test administration with the same participant offers the advantage of assessing changes in performance within the individual over time. However, repeated testing of the same domain may incur order effects. Practice effects can be defined as increments in performance across repeated administration in the absence of interventions (Bartels et al., 2010). Practice-induced increases in performance may be underpinned by reduced anxiety or increased familiarity with the testing environment, procedural learning or recall effects, general improvement in functions underlying test completion, or regression to the mean (Bartels et al., 2010; Benedict & Zgaljardic, 1998; McCaffrey & Westervelt, 1995). Decrements in performance are also possible, but less common and likely due to boredom or fatigue (McCaffrey, Ortega, & Haase, 1993).

The number of test presentations is an important factor in cognitive test practice effects. The distribution of practice effects appears to be loaded on early test presentations. The

largest increments are frequently reported between the first and second exposure (Beglinger et al., 2005; Collie, Maruff, Darby, & McStephen, 2003; Hausknecht, Halpert, Di Paolo, & Gerrard, 2007; Salthouse & Tucker-Drob, 2008). Performance increments thereafter are smaller in magnitude. The specific type of cognitive domain also influences the dynamics of practice effects. Executive control and learning and memory domains appear particularly vulnerable. However, test specificity is evident with some domain tests more vulnerable to practice effects than others (Bartels et al., 2010).

Whilst the complete elimination of practice effects from measures of cognitive performance over repeated administrations is unfeasible, a number of steps can be taken to reduce the potential influence. Firstly, alternative forms can be produced for tests that do not present test stimuli randomly. This can reduce potential learning and familiarity effects. Secondly, evidence of the dynamics of practice effects suggests over-exposing participants to tests may remove some of the effects of practice. Administering multiple initial exposures and employing the last exposure as baseline for subsequent assessment may cut off a proportion of the early practice-induced enhancement. This is proposed to increase the stability of the baseline performance level as the largest proportion of the practice effects occur during early test exposures (Bartels et al., 2010; Benedict & Zgaljardic, 1998; McCaffrey et al., 1993; McCaffrey & Westervelt, 1995).

All cognitive tests employed in this thesis presented stimuli in a random manner. Alternative forms of the task-switch test were produced to vary the colour of stimuli presented during each test exposure (described in Section 3.5.3.4). To establish baseline cognitive performance levels participants completed two initial test exposures. The subsequent third exposure was used as an established baseline for assessment. The VISGED test was the only exception. The VISGED was only administered once to establish a baseline due to the length of this test (9 – 12 minutes to complete).

3.5.2 Toronto Alexithymia Scale 20 Item Version (TAS-20)

The TAS-20 (Bagby, Parker, & Taylor, 1994) is a 20-item self-report measure of alexithymia (Appendix 14). The TAS-20 is comprised of three subscales of alexithymic impairment: *Difficulty Describing Feelings* (DDF; 7 items), *Difficulty Identifying Feelings* (DIFF; 5 items), and *Externally-Orientated Thinking* (EOT; 8 items). A total score can be calculated by summing all subscales. Respondents are required to indicate the extent to which each scale item describes them (e.g., “I am often confused about what emotion I am feeling”). Responses are made in reference to a five-point Likert scale: *strongly*

disagree = 1*, disagree* = 2*, neither agree nor disagree* = 3*, agree* = 4*,* and *strongly agree* = 5*.* Likert scoring is reversed for negatively weighted items. Reliability coefficients for total score range between r_{α} = .73 - .84; r_{tt} = .77 - .83 (Bagby et al., 1994; Rodrigo, Lusiardo, & Normey, 1989). Subscale reliability: DDF, r_{α} = .76 - .84; DIF, r_{α} = .73 - .84; and EOT, r_{α} = .59 - .71 (Bagby et al., 1994; de Timary et al., 2008; Parker, Taylor, & Bagby, 2003; Rodrigo et al., 1989).

3.5.3 Tests of Cognitive Performance

The tests of cognitive performance selected and piloted across the thesis are detailed here. Specific manipulations or adjustments to cognitive tests (e.g., task load, inter-stimulus delay) are outlined in the method sections of relevant experimental chapters. Cognitive tests employed in a naturalistic setting were administered on an Acer AS5940G laptop (screen size, 15.5"; screen resolution, 1280 x 800 pixels). Laboratory-based tests were administered on a Dell Optiplex 760 desktop computer (screen size, 17"; screen resolution, 1280 x 800 pixels).

3.5.3.1 N-back

The n-back is a continuous performance task that measures monitoring, manipulation, and updating working memory processes. The n-back is considered to measure an active part of WM function associated with the maintenance and dynamic rehearsal of information (Engle, Tuholski, Laughlin, & Conway, 1999). The task requires respondents to continuously monitor a stimulus sequence comprised of letters, numbers, or pictures and identify if each stimuli presented matches the stimuli presented *n* items back in the sequence or not. The load factor *n* can be adjusted to make the task more or less difficult whilst overall task procedures remain constant across conditions.

The n-back has been employed extensively in neuroimaging studies that have demonstrated increased task load results in reduced performance and increased activation of dorsolateral and inferior frontal regions of the PFC (Braver et al., 1997; Manoach et al., 1997; Ragland et al., 2002). The task can be considered to have face validity in as much as it requires respondents to maintain and update a dynamic rehearsal set whilst maintaining response to each stimulus (Kane, Conway, Miura, & Colflesh, 2007). The n-back has also been shown to be more closely related to other WM tasks that require information manipulation compared to tasks requiring predominantly simple rehearsal (Engle et al., 1999).

A computerised (Eprime) digit 2-back was employed here (see Figure 3.1 for stimulus configuration). A series of digits from 0 to 9 were presented in a quasi-random sequence in trial blocks of 50 stimuli. Participants were required to decide if the digit presented was a target (matched the digit presented *2* steps back) or a non-target (did not match the digit *2* steps back). Responses were made on a keyboard using the "1" key to record a target and the "2" key for a non-target stimulus. Presentation of all stimuli was random, negating the need for alternate parallel test versions. Target stimuli were presented randomly with a probability of 33%. The number of trial blocks presented was manipulated across studies (details provided in specific study methods sections). Stimuli were displayed for 500 ms with an inter-stimulus interval of 850 – 1000 ms (manipulated across studies). The first three stimuli in each trial block were not targets.

Four performance scores were calculated upon completion of the 2-back task: target accuracy (sum of correctly identified targets as a percentage of total number of targets), total accuracy (sum of correctly identified targets – number of false alarms [incorrect identification of a non-target as a target]) as a percentage of total number of targets, RT for identification of targets, and RT for identification of non-targets.

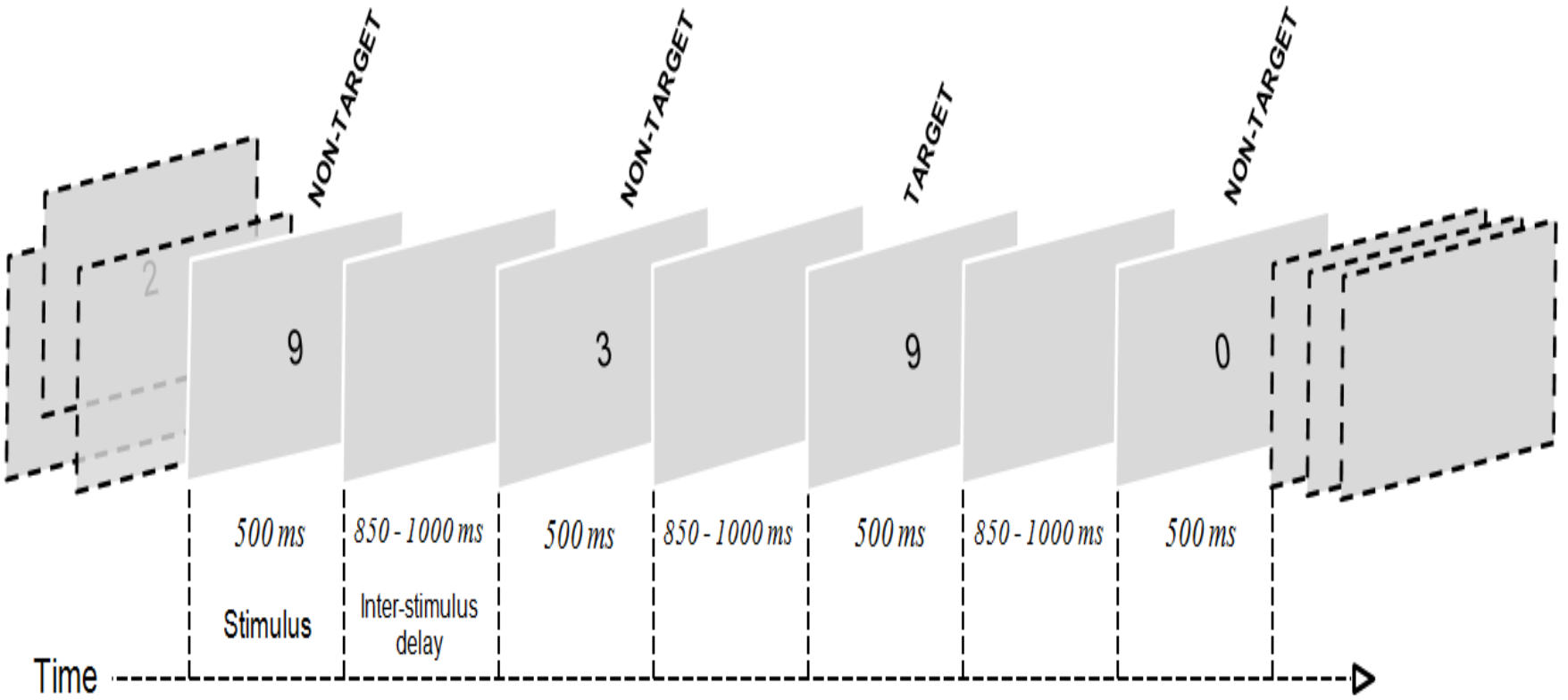


Figure 3.1 Stimulus configuration of the 2-back task with target and non-target stimuli labelled

3.5.3.2 Automated Operation Span (A-ospan)

Working memory span tasks, such as the counting span, operation span (ospan), and reading span, are among the most widely used tasks of cognitive performance (Conway et al., 2005). Span tasks of WM require participants to remember a string of stimuli (e.g., words or letters) when interspersed with a distracting task (e.g., completing maths operations or reading sentences). Target stimuli must then be recalled serially. Span tasks were developed from the perspective of Baddeley and Hitch's (1974) theory of WM that emphasises the function of WM as actively maintaining goal-relevant information in the service of more complex ongoing cognitive processes and tasks, rather than being solely involved in the passive storage and rehearsal of information. Span tasks place demands on information storage and rehearsal, but also simultaneous processing of additional information. Therefore, span tasks are considered to be measures of the capacity of WM (Daneman & Carpenter, 1980; Turner & Engle, 1989). Working memory capacity (WMC) is presumed to be closely associated with more complex, higher-order cognitive functions. Indeed, a strong relationship between performance on tests of WMC and fluid intelligence and general intellectual ability has been reported (Conway, Cowan, Bunting, Therriault, & Minkoff, 2002; Engle et al., 1999; Kyllonen & Christal, 1990; Unsworth & Engle, 2005). Span tasks have been shown to have good reliability and validity, independent of the type of distracting task or to-be-remembered stimuli used (Conway et al., 2002; Engle et al., 1999). High internal consistency and test-retest reliability has also been reported (Klein & Fiss, 1999).

A computer-based, mouse-driven automated version of the ospan task was employed here (Nash Unsworth, Heitz, Schrock, & Engle, 2005). The automated version of the ospan (A-ospan) removes the need for an experimenter and ensures standardization of test presentation. All instructions and sections of the test are presented on a computer screen using Eprime software. Participants were required to solve a series of maths operations whilst trying to remember a series of letters presented in between completion of each maths operation. At the end of each set of maths problems and letter presentations participants were required to recall the letters in serial order. The number of maths operations and letters for recall (set size) was manipulated to increase task load. Set sizes between 3 and 5 were employed across the thesis studies. The specific set sizes employed are reported in the method sections of each study presented in this thesis.

The participants completed a number of practice trials prior to completion of the A-ospan task trials. Practice trials were divided into three sections. First, participants completed four letter spans. A series of four letters were shown sequentially in the centre of the monitor. All letters (in practice and test trials) were shown on the screen for 800 ms. Respondents were required to recall the letters in serial order. Recall responses were made on a recall screen comprised of a 4 x 3 letter matrix (F, H, J, K, L, N, P, Q, R, S, T, and Y). Respondents were instructed to click the box next to the letters in the correct order. If respondents were unable to recall any letter in the sequence they were instructed to tick the box labelled “blank”. The recall phase was untimed. Feedback on letter recall accuracy was given after serial recall. Second, eight practice maths operations were completed. A maths operation was presented on the screen (e.g., [5 + 4] * 4 = ?) with instructions to solve the operation as quickly as possible and click the mouse button once solved. On the next screen, a digit was shown on the screen and the participant was required to click true or false to indicate if the digit was the correct answer to the maths operation. Practice maths operations were used to establish individual mean maths operation response time. To account for individual differences in time required to solve maths operations, the mean practice maths operation response time (plus 2 *SD*) was calculated and used as a time limit for trial maths operations. The limit of 2.5 *SD* was based upon extensive piloting undertaken by Unsworth et al. (2005). If respondents took longer than their mean response time (plus 2.5 *SD*) during trial maths operations, the program automatically moved to the letter screen and recorded that trial as an error. This was to reduce the opportunity for maths operation rehearsal. For the final section of the practice trials, participants completed four sets (two set size 3; two set size 4) of the letter recall and maths operations together as required in the actual trials. Task trials commenced upon completion of the practice trials (see Figure 3.2 for A-ospan stimulus configuration).

The order of trial set sizes, letters for recall, and maths operations and answers were presented randomly. In order to ensure that participants did not trade off between solving the maths operations and remembering the words they were asked to try to keep maths operation accuracy above 85%. Percentage maths accuracy feedback was shown on screen after each serial recall.

Four performance scores were generated upon completion of the A-ospan. *Ospan score* is a measure of absolute scoring performance calculated by summing all perfectly recalled sets. For example if a respondent recalls 3 letters in a set size of 3,

2 letters in a set size of 3, and 4 letters in set size of 4, this would generate a score of 7 (i.e., 3 + 0 + 4). *Ospan total* is calculated by summing the total number of letters recalled in the correct position irrespective of individual set accuracy. Two error types are calculated from performance on the maths operations: s*peed errors,* in which participants ran out of time to solve the operation, and *accuracy errors,* in which participants selected the incorrect answer to an operation.

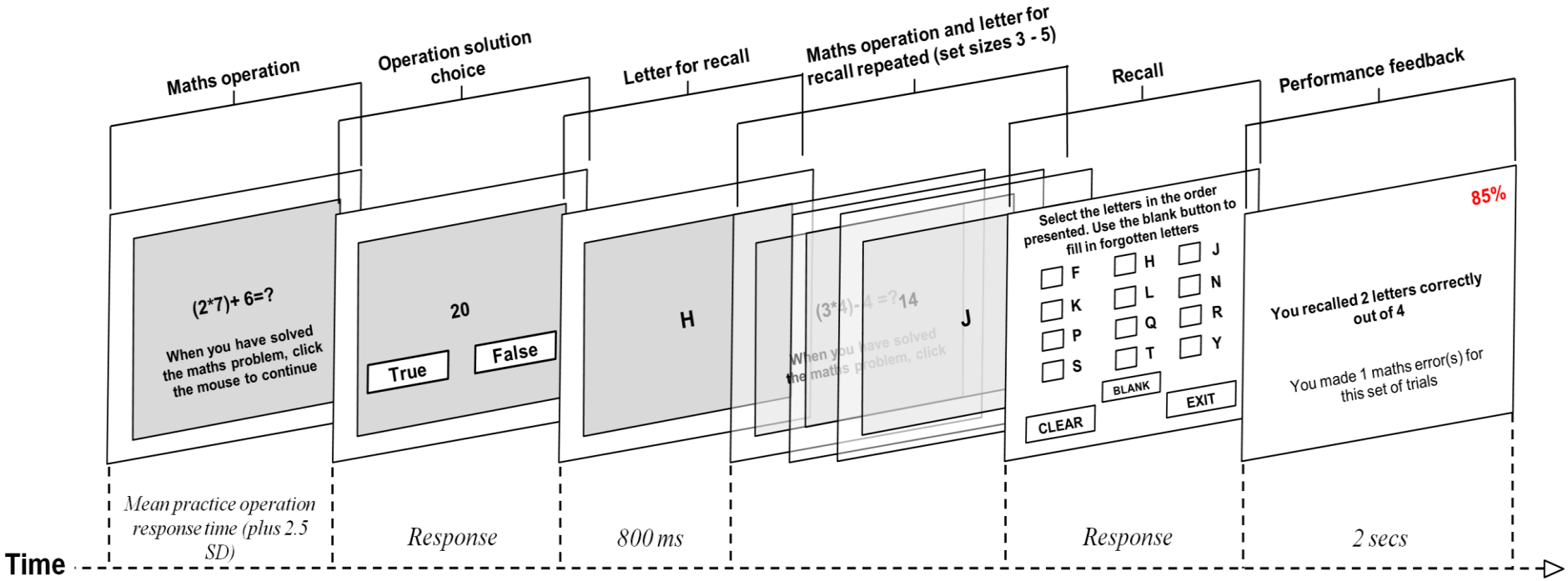


Figure 3.2 Trial stimulus configuration of the automated ospan task (A-ospan)

A maths operation is shown first (remaining on screen for mean response time (± 2.5 *SD)* established during practice trials [*n*=8]). Participants click once they have solved the operation and decide if the digit shown on the response screen is correct or incorrect. Next the to-be-remembered letter is shown for 800 ms. This sequence is repeated depending on the set size (3 – 5). After the set is complete, a serial response screen is shown. Participants are required to recall the letters from the set in serial order. Once the participant indicates they have completed the serial recall screen, performance feedback (maths accuracy and letters recalled) is shown for 2 sec. The next set then begins.

3.5.3.3 VISGED

The VISGED (Visueller Gedächtnistest [Eng. visual memory test]) is an adaptive test of visual memory performance developed by Etzel and Hornke (1999). This test is based upon principles of computerised adaptive testing (CAT) which adapt to the respondents' individual ability level. The test was designed to assess visual detection, memory, and recall of geographical landmark positions on a map. For each trial, participants are shown an on-screen city map marked with geographical landmark symbols (e.g., airport, train station, hospital etc.). The total number of trials presented, the complexity of the city map, the duration of stimulus presentation, and the numbers of symbols required to-be-remembered for each trial (up to a maximum of eight) varies depending upon the performance level of the respondent. Participants are required to memorise the location of the symbols for immediate recall after each trial. Recall is tested by presenting the city map without symbols and asking participants to mark on the map where each landmark symbol was located for that trial. The actual position of the landmark symbol is given after the recall and placement of each symbol to provide feedback on accuracy. Test screen shots are shown in Figure 3.3.

Computerised adaptive tests use an iterative algorithm to calculate the respondent's performance level. The VISGED selects the difficulty level of each trial based upon the performance of the individual. The first trial presented to all participants is of medium difficulty as no performance feedback is available prior to test administration. Subsequent trials are selected from a 126 item pool of varying task difficulty dependent upon the respondent's level of performance. The construction of the trials administered to each respondent is calculated using Rasch model item response theory (Rasch, 1980) wherein for a respondent (*i)* with a specific test aptitude (θ_i), the probability of answering an item (*j*) of a specific difficulty (β_j) correctly ($X_{ij} = 1$) can be calculated using Equation 1.5.

$$P(X_{ij} = 1) = \frac{e(\Theta_i - \beta_j)}{1 + e(\Theta_i - \beta_j)}$$

Equation 1.5 Item response theory equation for calculation of performance-mediated VISGED trial selection

Using this model, the probability of a specified response (i.e., correct or incorrect answer) can be modelled as a logistic function of the difference between the person and item parameter. Once the respondent's performance level is established (following completion of the first medium difficulty trial) subsequent trials are selected from the item pool based upon current performance. Estimates of performance are updated after each trial completion and used to select subsequent trials from the item pool. This performance-mediated presentation of trials is repeated until a test termination criterion is met wherein a sufficient amount of information needed to establish test performance level is reached. The VISGED calculates a "person parameter" that is a measure of visual memory performance. The VISGED uses the VIENNA TEST SYSTEM® platform for test presentation. Reliability coefficients ranging between r_{α} = .75 - .94 have been reported for the screening version administered here (Arendasy, Sommer, & Hergovich, 2007; Etzel & Hornke, 1999).

Figure 3.3 VISGED screenshots showing task instructions (screenshots 1 – 5) and an example trial (screenshot 6 – 8).

3.5.3.4 Task-switch test

Task-switch tests are measures of executive control. These tests assess an individual's ability to flexibly switch attention from one task to another according to contextual cues. The ability to switch between tasks is a fundamental function of executive control. Task-switch tests typically require respondents to repeatedly perform a task on some trials then switch to another task when prompted to do so by a cue. Performance level on repeated trials is typically superior to performance on "switch" trials; indexed by RT and accuracy. This decrement in performance is the *switch cost* which reflects the time and effort needed to switch between the two tasks. The task-switch test used in this thesis was based upon a test originally develop by Wylie, Javitt and Foxe (2003) that combines a task-switch paradigm with a Go/noGo task.

Letter-number pairs were presented on a horizontal plane in the centre of the screen for 1 sec (120 ms inter-stimulus). Each character was 1° to the left or right of the central fixation point (randomly determined). The letters were taken from a set containing 4 vowels (A, E, I, and U) and four consonants (G, K, M, and R). The numbers were taken from a set containing 4 even numbers (2, 4, 6, and 8) and 4 odd numbers (3, 5, 7, and 9). The letter-number pairs were presented in one of two alternating colours every three trials. Respondents were required to make a Go/noGo choice based upon the colour of the letter-number pairs. The change in colour cued the switch in task-set. For example, when the letter-number pairs were red, respondents were required to respond when the letter was a vowel (Go), but not when the letter was a consonant (noGo). Alternatively, when the letter-number pairs switched to blue, respondents were required to respond when the number was even (Go), but not when the number was odd (noGo). The three trials in each task-set can be split into: switch trials, nested and pre-switch trials. Switch trials are the first letter-number pairs presented after the task-switch (i.e., the Go/noGo colour switch). Nested and pre-switch are the subsequent repeat trials within the same task-set. Performance decrements are expected on switch trials compared to nested and pre-switch trials as executive control processes are required to switch between tasks. Nested and pre-switch trials are repetitions of the same task-set so require less executive control.

In total 144 trials were presented in a single trial block with target trials randomly presented with a probability of 50%. Responses were made by pressing the spacebar on a keyboard. Letter-number pairs were randomly selected for each trial but no

number or letter was the same as that presented in previous trial. Letter-number pair combinations were selected with equal probability. Consequently, an equal proportion of congruent pairs (letter and number mapped to same response; e.g., E 4) and incongruent pairs (letter and number mapped to different responses; e.g., E 9) were presented. Five versions of the task were employed differing only with respect to colours used to cue the task-switch. See Figure 3.4 for task-switch stimulus configuration.

Accuracy (number of correctly identified target letter-number pairs) and RT (time to respond to identify target letter-number pairs) across each trial type were measured. Accuracy and RT switch cost for switch and repeat trials were also calculated. Switch costs were determined by calculating the difference in accuracy and RT between contiguous trials using the nested trial as a comparator (switch trial cost switch: switch trial – nested trial; repeat trial switch cost: pre-switch trial – nested trial). The accuracy switch costs are presented as a percentage of the total number of targets of that type (e.g., switch trial – nested trial/total number of targets (72)*100).

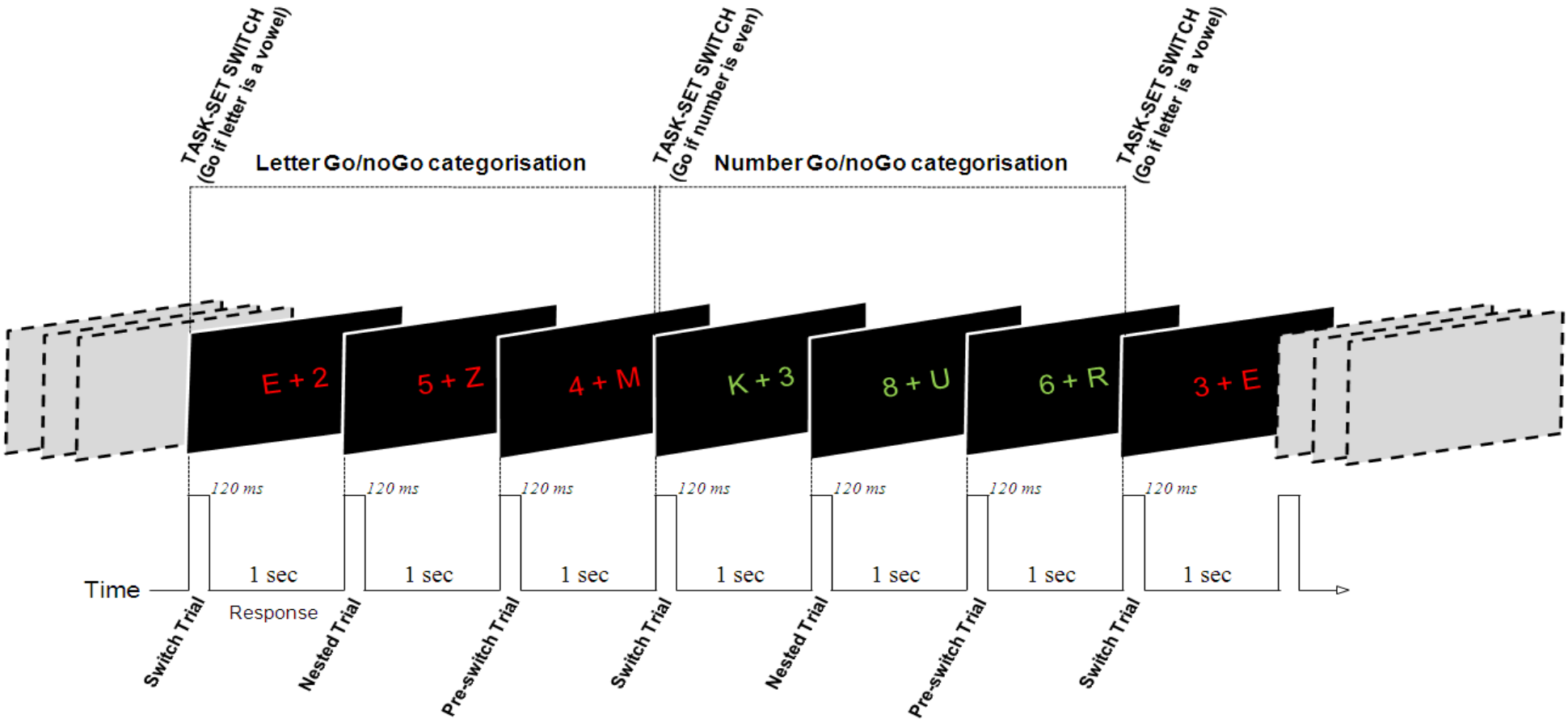


Figure 3.4 Stimulus configuration of the attention-switching task.

Task-set switch cues and trial type (switch, nested, and pre-switch trials) are shown. Figure adapted from Wylie, Javitt and Foxe (2003).

3.6 Statistical Approaches Common across Thesis Studies

All statistical analyses were performed using SAS (Statistical Analysis System, Version 9.2; SAS Institute, Inc., Cary, NC) or PASW (Version 20.0, SPSS Inc. Chicago). All data were summarised and screened for outliers. Residual plots were inspected for deviations from normality. Skewed data were normalised using appropriate transformations. Untransformed data are presented in figures for clarity throughout the thesis. For all analyses, the significance level was set at $\alpha = 5\%$. The nominal α level was adjusted for multiple post-hoc mean least squares mean comparisons using the Tukey-Kramer correction (Tukey, 1951). All results (including figures and tables) are presented as mean and standard error of the mean (*SEM*).

3.6.1 Regression Analysis to Identify Predictors of Cortisol Responsivity

To explore for potential relationships between personality traits and responsivity of the HPA axis, the following statistical approaches were employed across the studies presented in this thesis. Partial correlations (two-tailed) controlling for sex (Studies 1 and 2) and age were performed to explore relationships between personality trait measures and cortisol responsivity. Delta increase (peak post-stress – baseline), AUCi, and AUCg were calculated as measures of aggregated cortisol responsivity. Further exploratory regression analysis was undertaken on any personality trait variables that correlated with measures of cortisol response to identify potential predictors of cortisol responsivity to stress. To address potential associations between trait variables and cortisol responsivity, the following procedure was employed. Mean cortisol time point measures (e.g., – 20, – 10, + 10, + 20, + 30) and mean aggregated measures of cortisol response (AUCg, AUCi, and delta increase) were entered separately as outcome variables in hierarchical regression models. To control for sex (Studies 1 and 2), age, BMI, and chronic stress (PSS) prior to repeated stress exposure (Study 3), these variables were entered as predictors in the first step of all analyses. Identified potential trait predictors of cortisol responsivity were entered at the second step. Correlation and regression analyses were conducted using PASW software (Version 20.0, SPSS Inc. Chicago).

3.6.2 Analysis of Between- and Within-Subjects Effects

The SAS-mixed models procedure (PROC MIXED) was employed to examine within- and between-subjects change across the primary outcomes variables in studies presented in this thesis. PROC MIXED analysis uses a likelihood-based estimation method to estimate all unknown variance-covariance parameters (Jennrich & Schluchter, 1986). In contrast to PROC GLM analysis, covariates are permitted to vary within a subject and models can accommodate data that are missing at random. PROC MIXED analysis requires the selection of a covariance structure for each statistical model produced. A strategy for covariance structure selection provided by Wolfinger (1993) was adopted for statistical analyses in studies presented in this thesis. This entails the specification of a number of covariance structures (compound symmetry, autoregressive, variance components, and unstructured) and comparison of the generated information criteria for each model. The smaller the information criteria value the better the fit of the covariance structure. Three criteria were used to identify an appropriate covariance structure: Akaike's Information Criteria (AIC), AIC Corrected (AICC), and Bayesian Information Criteria (BIC). The compound symmetry covariance structure was determined to be best fit for the all modelled data and employed in mixed models procedures reported in this thesis. Once a covariance structure was selected for the data, non-significant fixed effects in the model were removed and the covariance fit reassessed.

Chapter 4:
Study 1

Chapter 4 Study 1 - Stress Responses to a Naturalistic Competitive Audition Context Characterised by Social-evaluative Threat

4.1 Introduction

4.1.1 Identification of a Naturalistic Social-evaluative Stress Context

As discussed in Chapter 1, the capacity of psychosocial stressors to provoke the hypothalamic-pituitary-adrenal axis has been demonstrated to vary depending upon a number of psychological factors. Laboratory stressors characterised by social-evaluative threat and uncontrollability have been shown to be the most efficacious in the elicitation of a cortisol response (Dickerson & Kemeny, 2004). Stress protocols such as the TSST have been widely utilised in the examination of a wide range of biological and psychological stress response parameters in a standardised, controlled context. Laboratory stressors, although characterised by social-evaluative threat, tend to utilise an imagined goal (e.g., present yourself as a candidate for a fictitious job). However, an imagined goal is unlikely to be as intrinsically linked to an individual's real-world aspirations and feelings of self-worth as a naturalistic goal. Furthermore, the perception of threat to one's social status may not be perceived as publicly salient considering the anonymity assured during research participation. Rohleder et al. (2007) suggest that perception of, and response to, a social-evaluative stressor is more likely under conditions in which a central goal and demonstration of a skill valued by the individual are present. This emphasises the need for the identification of real-world stressors more salient to, and aligned with, the self-identity of the individual than the imagined goals utilised in laboratory stressors. Moreover, evidence suggests only moderate correlations between stress responsivity in the laboratory and real-world may exist (Johnston, Tuomisto, & Patching, 2008; Lundberg, Melin, Fredrikson, Tuomisto, & Frankenhaeuser, 1990; Van Doornen & Van Blokland, 1992; Van Eck et al., 1996).

Involvement in a competitive performance offers an opportunity to examine stress responses to an activity that involves a genuine personal goal and display of a skill valued and evaluated by others - central tenets of the social self-preservation theory. Additionally, Rohleder et al. (2007) demonstrated that this context (competitive ballroom dancing) was sufficient to provoke significant cortisol responses in the

majority of individuals over repeated exposures. Due to the constraints of the naturalistic testing environment, Rohleder et al. were limited to collecting brief subjective state measures from participants. Salivary cortisol samples could only be collected during the intervals of repeated dance performances over a tournament lasting approximately one to one and a half hours, and no cardiovascular measures were recorded.

A naturalistic context related to that proposed by Rohleder et al. that offers a number of potential additional advantages is participation in auditions for theatre productions. Participation in a performing arts audition shares many of the key elements of social-evaluative threat present in a competitive ballroom dancing contest. Performers demonstrate a valued skill, intrinsically linked to self-identity, in front of an evaluative panel with the goal of being selected for the performance role in preference to other auditionees. Successful performance in the audition is threatened by the evaluation of others and may be impeded by uncontrollable factors (e.g., the performance of other auditionees, audition location, and quality of venue). The performance arts audition format is more closely related to the TSST than a ballroom dancing contest in terms of psychological demands: performance of a motivated task in front of an evaluative panel; and structure: anticipation period, a single acute social-evaluative stressor exposure, and a recovery period. Furthermore, in contrast to laboratory stress protocols, this context comprises real-world social-evaluative threat that is intrinsically linked to self-perception and identity. The format of the audition context also allows for completion of more comprehensive subjective and physiological response measures following a single acute stress exposure.

4.2 Study Objectives and Hypotheses

Given the difficulties inherent in the collection of subjective and physiological measures in naturalistic settings, Study 1 explored the feasibility of utilising the performance audition context as a naturalistic acute stressor characterised by social-evaluative threat in future intervention study designs. Considering the heterogeneous capacity of different stress contexts to provoke acute elevations in cortisol response, the primary objective was to ascertain whether this context would be sufficient to elicit a cortisol response. This is an important consideration due to evident variability in the capacity of naturalistic stress contexts to provoke acute cortisol responses (Biondi & Picardi, 1999). Evidence of the importance of synergistic adrenergic and glucocorticoid arousal in the moderation of cognitive performance under stress

(Elzinga & Roelofs, 2005; Tollenaar, Elzinga, Spinhoven, & Everaerd, 2008) emphasises the need to identify a stress context that also activates adrenergic stress responses. Exposure to the audition context was expected to induce significant elevations in salivary cortisol and cardiovascular responsivity above baseline levels. Furthermore, this cortisol increase was expected to be associated with elements of social-evaluative threat. The identification of a sample with a tendency towards high cortisol responsivity was identified as a key objective of the subsequent nutritional intervention study. Therefore, psychometric trait personality measures previously associated with cortisol responsivity (previously discussed in Section 1.3.2.1) were collected to undertake exploratory analyses to identify potential predictors of cortisol response.

4.3 Methods

4.3.1 Sample

Sixteen (8 male; 8 female) healthy adults undertaking performance auditions participated (see Table 4.1 for sample characteristics). Participants were recruited by contacting local amateur dramatic performance groups. Eligibility was determined by screening questionnaires completed at an audition rehearsal meeting. All female participants were taking OC's at the time of testing.

4.3.2 Inclusion and Exclusion Criteria

Participants were recruited using the following criteria.

4.3.2.1 Inclusion criteria

- Male or female over 18 years of age

4.3.2.2 Exclusion criteria

As reported in Methodologies Section 3.3.4.

4.3.3 Design

The study conformed to a naturalistic design assessing repeated measures of endocrine, cardiovascular and subjective responses in individuals undertaking a performing arts theatre audition.

4.3.4 Endocrine Measures

4.3.4.1 Cortisol

Salivary cortisol samples were collected at – 20, – 10, + 10, + 20 and + 30 minutes relative to audition onset. Salivary-free cortisol concentrations were determined using a Salivary Cortisol Enzyme Immunoassay kit (EIA; Sarstedt; Nümbrecht, Germany; described in Section 3.2.1.1). Intra- and inter-assay variability was below 9.5 and 12.3% respectively.

4.3.5 Physiological Measures

4.3.5.1 Blood pressure

An Omron M7 ambulatory blood pressure monitor was used to measure SBP and DBP at – 20, – 10, + 10, + 20 and + 30 minutes relative to audition onset.

4.3.6 Screening Measures

4.3.6.1 Hospital Anxiety and Depression Scale (HADS)

Participants completed the HADS five days prior to the audition day (General Methodologies Section 3.3.2).

4.3.7 Subjective Measures

The following measures were employed to assess baseline chronic stress level and acute subjective responses to the audition. More detailed descriptions of measures are given in the General Methodologies Section where indicated.

4.3.7.1 Perceived Stress Scale (PSS)

The PSS (Cohen et al., 1983) was used to assess self-reported chronic stress during the month prior to the audition (General Methodologies Section 3.3.1). Participants completed the PSS five days prior to the audition day.

4.3.7.2 Social-evaluative threat visual analogue scales (VAS)

As a measure of subjective social-evaluative threat participants were asked to indicate how stressful/threatening they found aspects of the audition process using 100 mm

visual analogue scales (VAS) anchored by the descriptors "*totally disagree"* to "*totally agree"* (Appendix 17). Seven statements relating to elements of social-evaluative threat were completed – 10 minutes prior to the audition (e.g., importance of the audition performance to personal identity and self-esteem, concern over being judged negatively based upon performance, and worry over factors outside of their control affecting performance). This measure was based upon the social-evaluative VAS employed by Rohleder et al. (2007) to assess social-evaluative stress perceptions relating to a ballroom dancing competition. The descriptors were altered to match the context specific social-evaluative elements of a performing arts theatre audition.

4.3.7.3 Stress and Arousal Checklist (SACL)

The SACL was completed at – 10, + 10, + 20 and + 30 minutes relative to audition onset (General Methodologies Section 3.2.3.1).

4.3.7.4 Profile of Mood States (POMS)

The POMS was completed at – 10, + 10, + 20 and + 30 minutes relative to audition onset (General Methodologies Section 3.2.3.2).

4.3.7.5 Primary Appraisal Secondary Appraisal (PASA)

The PASA was completed – 20 minutes relative to audition onset (General Methodologies Section 3.2.3.4). Acceptable reliability coefficients were revealed for both *primary appraisal* (r_α = .69) and *secondary appraisal* (r_α = .89) scales.

4.3.8 Trait Personality Measures

The following trait personality measures were employed in order to explore potential trait predictors of acute cortisol responsivity to stress. More detailed descriptions of all personality measures are given in the General Methodologies (sections shown in parentheses). The reliability coefficients for each measure are given here. All personality measures were completed five days prior to auditions.

- Frost Multidimensional Perfectionism Scale (Section 3.4.1). Acceptable reliability coefficients were revealed for subscales: *Parental Expectation* (r_α = .78), *Organisation* (r_α = .89), *Parental Criticism* (r_α = .83), *Concern over Mistakes* (r_α = .77), *Personal Standards* (r_α = .77), and *Total Perfectionism Score* (r_α = .88). The subscale *Doubts about Actions* was shown to be less

reliable (r_α = .59). Less consistent reliability coefficients have previously been reported for this subscale (e.g. r_α = .66; Harvey et al., 2004).

- Rotter's external-internal Locus of Control Scale (Section 3.4.2; r_α = .82).
- Rosenberg Self-Esteem Scale (Section 3.4.3; r_α = .72)
- Neuroticism (IPIP-N; Section 3.4.4; r_α = .68)

4.3.9 Procedure

Participants were undertaking amateur stage auditions for musical theatre production principle performance roles. Successful auditionees would perform in a musical production at a local city theatre. All auditionees were members of amateur dramatic societies and received no monetary reward if successful in gaining a role. Auditions were undertaken in front of a panel of three evaluative judges in a theatre auditorium setting. Each auditionee was required to perform a short scripted spoken scene followed by a performance of a song in front of the selection panel judges. Testing occurred between 1145 and 1700 hrs. Participants were asked to refrain from exhaustive exercise, consuming meals or caffeinated/low pH drinks, and brushing teeth 1 hour prior to testing. Testing was undertaken in a rehearsal waiting room at the audition venue. Each participant reported to the experimenter 20 minutes prior to being called into the audition to complete pre-audition physiological and subjective measures. The Omron M7 blood pressure monitor was fitted whilst participants completed experimental measures but removed for the audition performance. All auditions lasted between 8 and 10 minutes. Upon completion of the audition, participants returned immediately to the rehearsal waiting room to complete post-audition measures. All measures were taken whilst participants were seated. A procedural timeline of the study is shown in Figure 4.1.

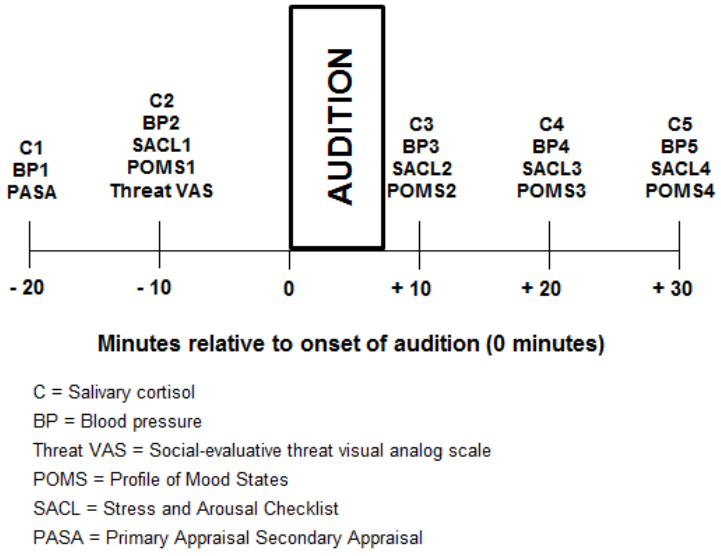


Figure 4.1 Procedural timeline

4.3.9.1 Ethical Approval

All participants provided written informed consent one week prior to study inclusion. The experimenter attended a rehearsal meeting to outline the study and give a participant information sheet to potential participants. Given the potentially stressful nature of audition participation the experimenter verbally reiterated the purpose and procedures of study participation to interested performers. Particular emphasis was given to the participants' right to withdraw from the study at any time before or during study participation. The study was approved by the University of Leeds' Institute of Psychological Sciences Research Ethics Committee (Ref: 10 068 – 11; submitted and approved April 2010). All participants were paid a £5 honorarium on study completion.

4.3.10 Statistical Analysis

Cortisol data were positively skewed and normalized using a logarithmic transformation. Independent T-tests were employed to compare participant characteristics across sex. The SAS-mixed models procedure (PROC MIXED) was employed to examine the within-subjects change in stress response outcome variables across audition exposure. Participant ID was entered as a random factor; time and sex were entered as fixed factors. Age, BMI, PSS score were also initially included as covariates but subsequently removed from models due to non-significance across all measures.

Partial correlations (two-tailed) were employed to explore the relationship between social-evaluative threat VAS ratings and cortisol responses, and subjective stress ratings (SACL) and cortisol and cardiovascular responses. The statistical approach employed for exploratory regression analyses is described in General Methodologies Section 3.6.1). The method of selecting covariance structure for the mixed models is described in General Methodologies Section 3.6.2.

4.4 Results

4.4.1 Participant Characteristics

Independent T-tests revealed no significant differences between males and females in terms of age, $t(14) = 0.15$, $p = .89$, BMI, $t(14) = -1.01$, $p = .33$, PSS score, $t(14) = -1.16$, $p = .26$, HADS-D, $t(14) = 0.19$, $p = .85$, or HADS-A, $t(14) = -0.55$, $p = .59$. Participant characteristics are shown in Table 4.1.

Table 4.1 Participant characteristics by sex (*N* = 16)

	Males		Females	
	$\overline{X}$ (SEM)		$\overline{X}$ (SEM)	
Age	25.88	(1.72)	25.50	(1.94)
BMI (kg/m²)	23.52	(0.69)	24.70	(0.94)
PSS	16.38	(1.75)	19.38	(1.89)
HADS-D	3.63	(0.38)	3.50	(0.53)
HADS-A	3.00	(0.50)	3.38	(0.46)

PSS - Perceived Stress Scale (score range 0-40).
HADS - Hospital Anxiety and Depression Scale (score range 0 - 21 for depression and anxiety scales).

4.4.2 Salivary Cortisol Response

Exposure to the competitive audition stressor provoked a significant salivary cortisol response reflected in a main effect of time, $F(4, 59) = 9.20$, $p < .001$ (Figure 4.2). Mean salivary cortisol levels were significantly higher at + 10, + 20, and + 30 minutes compared to mean pre-audition levels at – 20 and – 10 minutes (all comparisons significant at $p < .01$). The mean salivary cortisol level increase from baseline (– 20

minutes = 4.95 nmol/L) to peak (+ 20 minutes = 12.54 nmol/L) following audition exposure was 7.59 nmol/L, representing an increase of 153%. A trend for greater cortisol arousal in female participants compared to males was shown, but these sex differences did not reach significance, $F(1, 14) = 3.79$, $p = .07$.

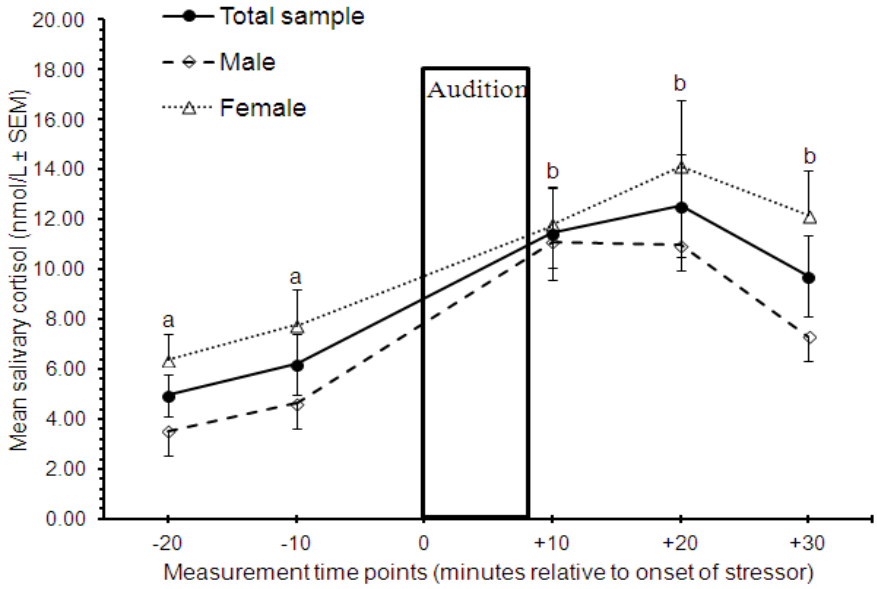


Figure 4.2 Mean (± *SEM)* salivary cortisol response (nmol/L) pre + post audition in the total sample and by sex. For total sample, *means* with different letters are significantly different from each other (all p < .01)

4.4.3 Cardiovascular Response

4.4.3.1 Systolic blood pressure

A significant time×sex interaction, $F(4, 56) = 4.24$, $p = .004$, for SBP responses across the audition exposure was revealed. Analysis also revealed main effects of time, $F(4, 56) = 5.93$, $p < .001$, and sex, $F(1, 14) = 40.25$, $p < .001$ (see Figure 4.3).

Across the total sample SBP was significantly higher at – 10 and + 10 minutes after onset of audition exposure compared to – 20 minutes prior to the audition. The SBP increase remained evident immediately after the audition with SBP significantly higher at + 10 compared to – 10 minutes. The post-stressor SBP response profile suggests some recovery toward baseline BP parameters by + 20 minutes. Mean SBP was significantly lower at + 20 and + 30 minutes, after the audition onset compared to the

peak response at + 10 minutes after audition onset (all significant at $p < .04$). However, the significant sex×time interaction was reflective of the significant increases in SBP response being attributable to male participants. Males demonstrated significantly higher SBP responses than females across all sampling time points (all significant at $p < .04$). Male SBP was significantly higher at + 10 minutes compared to – 20, – 10 and + 30 minutes (all significant at $p < .04$). No significant differences were revealed across the female SBP response profile.

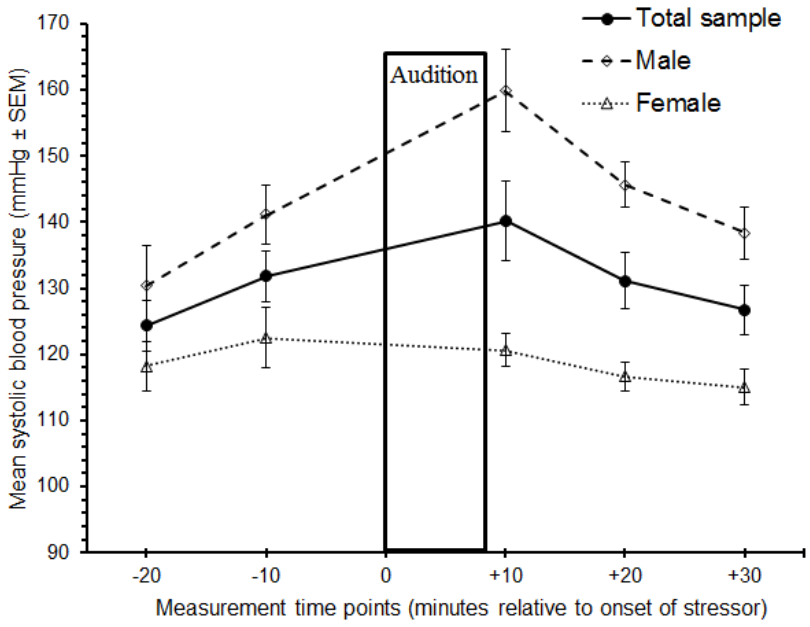


Figure 4.3 Mean (± *SEM)* SBP response (mmHg) pre + post audition exposure in the total sample and by sex

4.4.3.2 Diastolic blood pressure

Exposure to audition stress provoked significant changes in DBP reflected in a main effect of time, $F(4, 56) = 8.65$, $p < .001$ (Figure 4.4). Mean DBP was significantly higher – 10, + 10, + 20, and + 30 minutes compared to – 20 minutes. Diastolic BP was also significantly higher at + 10 compared to – 10 minutes. A pattern of recovery towards baseline levels shown in SBP was also shown for DBP. Mean DBP was significantly lower at + 30 minutes after the audition onset compared to peak response at + 10 (all significant at $p < .03$).

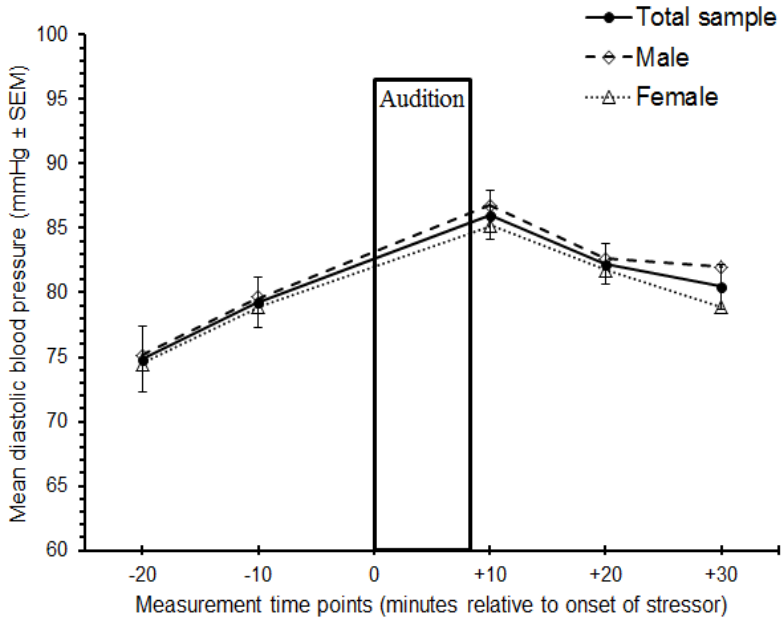


Figure 4.4 Mean (± *SEM)* DBP response (mmHg) pre + post audition exposure in the total sample and by sex

4.4.4 Subjective Response

4.4.4.1 Stress and Arousal Checklist

Audition stress exposure had a significant effect on subjective stress and arousal reflected in a main effect of time for SACL stress, $F(3, 42) = 12.82$, $p < .001$, and arousal, $F(3, 42) = 4.89$, $p < .001$, ratings The *means* (± *SEM*) of subjective stress and arousal ratings are shown in Table 4.2. Participants reported feeling significantly higher levels of subjective stress – 10 and + 10 minutes compared to + 20 and + 30 minutes relative to audition onset. A decline in subjective stress rating was evident after 30 minutes with significantly lower ratings at + 30 compared to + 20 minutes. Subjective ratings of arousal were significantly higher at – 10 and + 10 minutes compared to + 20 and + 30 minutes (all significant at $p < .02$).

Table 4.2 Mean (± *SEM)* subjective stress/arousal (SACL) and mood (POMS) responses pre + post audition exposure. Score ranges are shown in parentheses next to each measure dimension

	Time in minutes relative to audition onset							
	- 10		+ 10		+ 20		+ 30	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
SACL								
Stress (18 - 72)	**49.80**	2.90	**50.30**	3.00	**43.80**	2.98	**38.80**	2.41
Arousal (12 - 48)	**39.10**	1.21	**39.40**	1.10	**34.90**	2.01	**34.00**	1.99
POMS								
Tension-anxiety (0 - 36)	**22.75**	2.11	**15.81**	1.51	**11.63**	1.70	**10.06**	1.65
Depression-dejection (0 - 60)	**5.31**	1.13	**6.75**	2.01	**5.69**	1.72	**5.00**	1.52
Anger-hostility (0 - 48)	**3.06**	0.79	**2.94**	0.64	**2.50**	0.63	**2.50**	0.84
Vigour-activity (0 - 32)	**15.75**	1.12	**16.38**	1.75	**12.81**	1.88	**10.81**	2.13
Fatigue-inertia (0 - 28)	**3.63**	0.97	**5.94**	1.35	**5.69**	1.43	**5.38**	1.86
Confusion-bewilderment (0 - 28)	**10.88**	1.35	**9.69**	1.40	**7.06**	1.36	**6.06**	0.99
TMD (0 - 200)	**45.63**	5.40	**41.13**	6.66	**32.57**	5.57	**29.00**	5.50

SACL = Stress and Arousal Checklist; POMS = Profile of Mood States; TMD = Total Mood Disturbance

Highlighted sections denote significant differences across the response profile

4.4.4.2 Profile of Mood States

Audition stress exposure had a significant effect on subjective mood ratings reflected in main effects of time for ratings of Tension-anxiety, $F(3, 42) = 15.95$, $p < .001$, Confusion-bewilderment, $F(3, 42) = 10.20$, $p < .001$, Vigour-activity, $F(3, 42) = 7.45$, $p < .001$, and TMD score, $F(3, 42) = 4.01$, $p = 0.01$. Non-significant effects of audition exposure were revealed for ratings of Depression-dejection, $F(3, 42) = 1.06$, $p = .38$, Anger-hostility, $F(3, 42) = 0.33$, $p = .08$, and Fatigue-inertia, $F(3, 42) = 1.45$, $p = .24$.

All mean ratings (± *SEM*) are shown in Table 4.2. Mean rating of subjective Tension-anxiety was significantly higher at – 10 minutes pre-audition compared to post-audition ratings at + 10, + 20, and + 30 minutes, and significantly higher at + 10 minutes compared to + 20 and + 30 minutes (all significant at $p < .04$). Mean subjective ratings of Vigour-activity and Confusion-bewilderment were significantly higher at – 10 and + 10 minutes compared to ratings at + 20 and + 30 minutes relative to audition onset (all significant at $p < .03$). The TMD score was significantly higher at – 10 minutes pre-audition compared to + 20 and + 30 minutes post-audition onset (both significant at $p < .001$).

4.4.5 Predictors of Cortisol Responsivity

4.4.5.1 Associations between trait variables and cortisol response

Partial correlations (two-tailed) controlling for sex and age revealed Perfectionism: Organisation to be the only trait variable significantly correlated with measures of cortisol response. Perfectionism: Organisation significantly correlated with AUCg, $r(12) = .60$, $p = .02$, AUCi, $r(12) = .65$, $p = .01$, and cortisol measures collected at + 10, $r(12) = .73$, $p = .003$, and + 20, $r(12) = .63$, $p = .02$, minutes relative to audition onset.

Exploratory regression analysis revealed the control variables (age, sex, and BMI) did not significantly predict AUCg (step one). The inclusion of Perfectionism: Organisation at step two resulted in a model that accounted for 40% of the adjusted variance in salivary cortisol AUCg. Perfectionism: Organisation was the only significant predictor in the model and increased the adjusted explained variance by 39%. The AUCi regression model did not reach significance ($p = .08$; see Table 4.3 for aggregated cortisol regression values).

Analysis also revealed the control variables (age, sex, and BMI) did not significantly predict cortisol levels at + 10 and + 20 minutes (step one). The inclusion of Perfectionism: Organisation at step two resulted in models that accounted for 47% (+ 10 minutes) and 44% (+ 20 minutes) of the adjusted variance in salivary cortisol. Perfectionism: Organisation was the only significant predictor in both models and increased the adjusted explained variance by 31% and 44% respectively (regression values shown in Table 4.4). For illustrative purposes the cortisol response profiles for high and low Perfectionism: Organisation (based on a median split) are shown in Figure 4.5.

Table 4.3 Hierarchical multiple regression analyses of relationships between Perfectionism: Organisation and aggregated measures of salivary cortisol (controlling for sex, age, and BMI)

		AUCg					*AUCi*			
		B	*SE B*	*β*	*F* change		*B*	*SE B*	*β*	*F* change
Step 1										
	Constant	58.92	57.37			Constant	55.86	58.15		
	Sex	13.76	10.12	.36		Sex	-1.04	10.26	-.03	
	Age	0.86	1.01	.22		Age	0.32	1.02	.09	
	BMI	-2.67	2.24	-.32	1.05	BMI	-1.82	2.27	-.23	0.28
Step 2										
	Constant	30.49	45.73			Constant	26.30	45.38		
	Sex	8.71	8.07	.23		Sex	-6.29	8.01	-.18	
	Age	0.84	0.79	.21		Age	0.3	0.78	.08	
	BMI	-3.05	1.75	-.36		BMI	-2.21	1.73	-.28	
	Perfectionism Org	2.09	0.71	.61*	8.76*	Perfectionism Org	2.17	0.70	.68*	9.61*

AUCg Step 1: $R^2 = .21$, $\Delta R^2 = .01$, $F(3,12) = 1.05$, $p = .41$; Step 2: $R^2 = .56$, $\Delta R^2 = .40$, $F(4,11) = 3.49$, $p = .04$.
AUCi Step 1: $R^2 = .07$, $\Delta R^2 = -.17$, $F(3,12) = .28$, $p = .84$; Step 2: $R^2 = .50$, $\Delta R^2 = .32$, $F(4,11) = 2.76$, $p = .08$.
* $p < .01$
Perfectionism Org - Organisation

Table 4.4 Hierarchical multiple regression analyses of relationships between Perfectionism: Organisation and salivary cortisol measures (controlling for sex, age, and BMI)

		Measurement time point (relative to audition onset)								
		+ 10 minutes					+ 20 minutes			
		B	*SE B*	*β*	*F* change		*B*	*SE B*	*β*	*F* change
Step1										
	Constant	4.36	3.45			Constant	5.19	3.43		
	Sex	0.45	0.61	.21		Sex	0.82	0.60	.36	
	Age	0.02	0.06	.09		Age	0.04	0.06	.17	
	BMI	-0.10	0.13	-.21	0.3	BMI	-0.17	0.13	-.34	1.01
Step 2										
	Constant	2.40	2.37			Constant	3.41	2.62		
	Sex	0.10	0.42	.05		Sex	0.51	0.46	.22	
	Age	0.02	0.04	.08		Age	0.04	0.05	.16	
	BMI	-0.12	0.09	-.26		BMI	-0.19	0.10	-.39	
	Perfectionism Org	0.14	0.04	.76*	15.53*	Perfectionism Org	0.13	0.04	.64**	10.45**

+ 10 minutes Step 1: $R^2 = .07$, $\Delta R^2 = -.16$, $F(3,12) = .30$, $p = .82$; Step 2: $R^2 = .61$, $\Delta R^2 = .47$, $F(4,11) = 4.38$, $p = .02$.
+ 20 minutes *Step 1:* $R^2 = .20$, $\Delta R^2 = .001$, $F(3,12) = 1.01$, $p = .42$; Step 2: $R^2 = .59$, $\Delta R^2 = .44$, $F(4,11) = 3.96$, $p = .03$.
* $p < .002$, ** $p < .008$
Perfectionism Org - Organisation

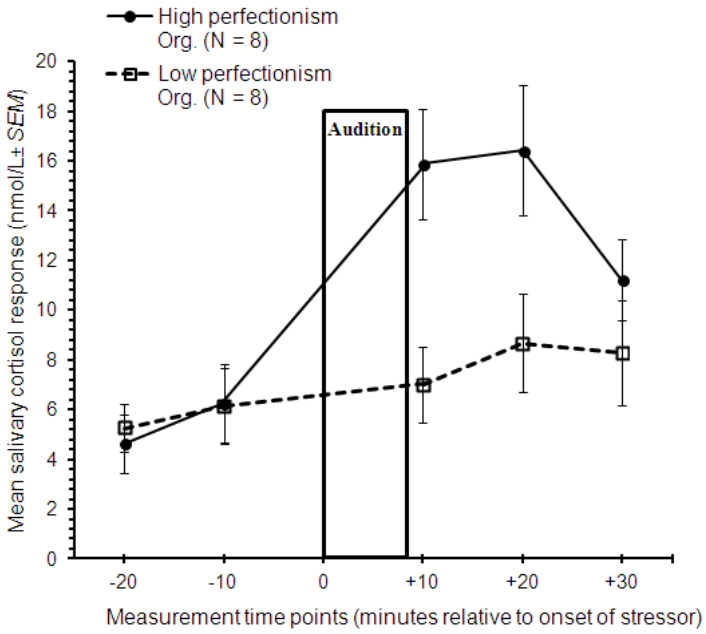


Figure 4.5 Mean (± *SEM)* salivary cortisol response (nmol/L) pre + post audition exposure in high and low Perfectionism: Organisation participants (based on a median split)

4.4.6 Subjective Stress and Physiological Stress Response

Partial correlations (two-tailed) revealed significant relationships between subjective ratings of stress (SACL) at + 20 minutes and cortisol AUCi, $r(12) = .54$, $p = .05$, and salivary cortisol level at + 20 minutes, $r(12) = .70$, $p = .005$. Subjective rating of stress at – 10 minutes was significantly related to DBP at – 10 minutes, $r(12) = .66$, $p = .01$, prior to audition onset.

4.4.7 Social-evaluative Threat and Cortisol Response

Partial correlations (two-tailed) controlling for sex and age revealed significant relationships between the extent to which participants believed they would be judged negatively if they gave a poor performance (social-evaluative threat VAS) and cortisol AUCi, $r(12) = .73$, $p = .003$, cortisol delta increase, $r(12) = .72$, $p = .004$, and salivary cortisol levels at + 10, $r(12) = .59$, $p = .03$, and + 20, $r(12) = .72$, $p = .004$, minutes after the onset of audition exposure (see Figure 4.6 for scatterplots).

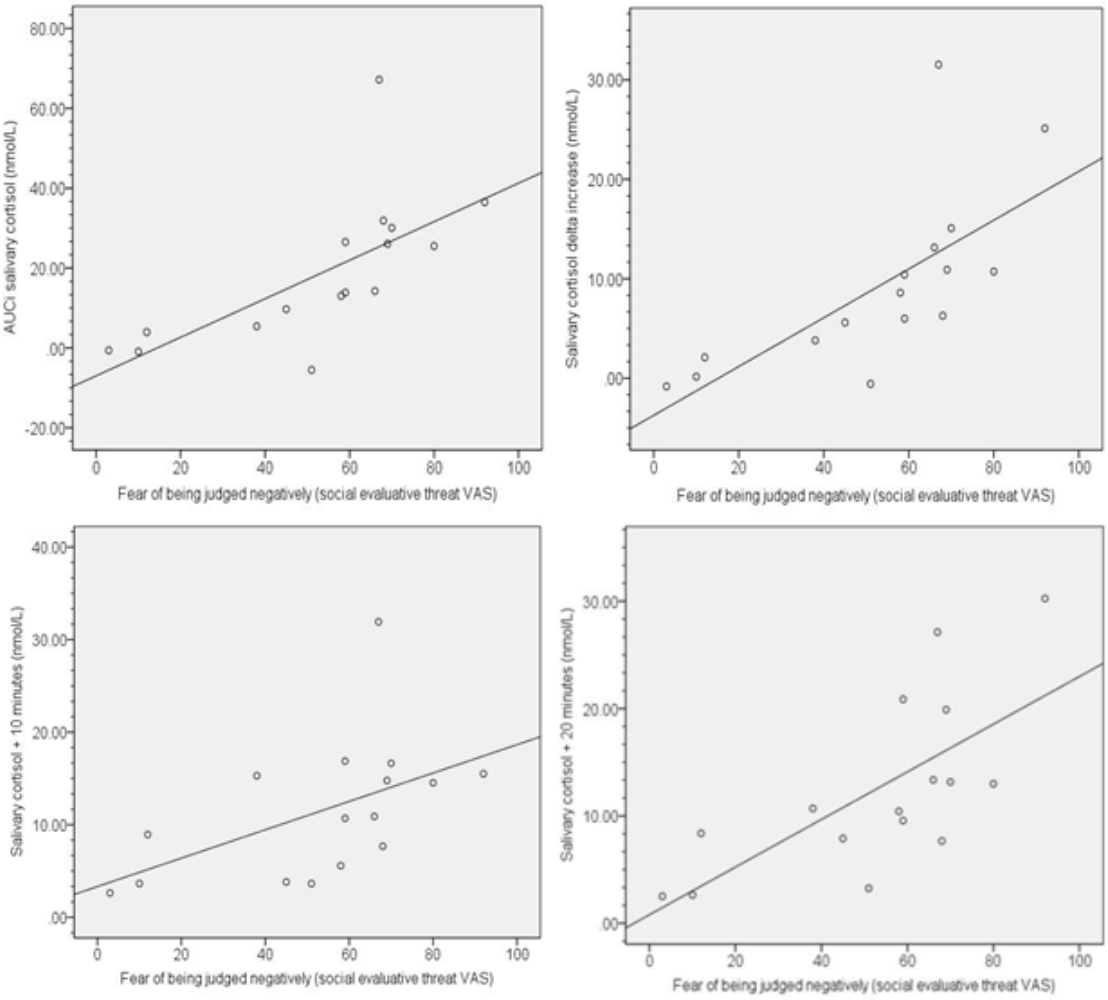


Figure 4.6 Scatterplots of fear of being judged negatively (social-evaluative threat VAS) rating and salivary cortisol AUCi, delta increase, and measurements at + 10 and + 20 minutes relative to audition onset (nmol/L)

4.5 Discussion

4.5.1 Salivary Cortisol Response

The results of Study 1 support the hypothesis that an audition context is sufficient to activate the HPA axis and provoke a significant acute salivary cortisol response. The peak cortisol increase of 153% is less than the two-threefold increase often reported in TSST studies (Dickerson & Kemeny, 2004; Kudielka, Hellhammer, & Wust, 2009), but towards the uppermost range of the increase previously reported in naturalistic contexts (0 – 180%; Michaud et al., 2008). In the present study a non-significant trend

for greater cortisol response was found in females. Typically, greater cortisol responsivity to psychosocial stress protocols such as the TSST is demonstrated by young males (Kudielka & Wust, 2010) Furthermore, higher cortisol levels in female participants are surprising since all were taking oral contraceptives (OC's) which have been shown to dampen HPA axis reactivity (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Lower basal cortisol levels in male participants may have contributed to this differentiation.

One advantage of a naturalistic competitive performance stress context is the potential for limited habituation across repeated exposures. The identification of a stress context with reliable and comparable perturbance in cortisol activation across repeated exposures was identified as a desirable objective for the planned intervention study. Rohleder et al. (2007) reported no habituation in cortisol responses across three consecutive competitive dance contests and no moderation of cortisol responsivity by level of previous experience. Participants completing auditions were asked to indicate their level of previous audition experience in order to allow analysis of moderation of response by experience. However, it became clear that participants were unable to provide an adequately precise estimate which would permit their categorisation based on previous audition experience.

4.5.2 Cardiovascular Response

Significant increases in arterial blood pressure, peaking after the cessation of the audition, also indicate that the audition context represented a significant challenge for individuals. Significant increases in DBP, independent of sex, were revealed. For SBP this effect was only observed in male participants. This effect could be expected since greater male cardiovascular reactivity to stress, specifically greater SBP, is often demonstrated in psychosocial stress protocols (Matthews, Gump, & Owens, 2001; Steptoe et al., 1996). Interestingly, this response divergence appears to emerge during adolescence (Matthews & Stoney, 1988). The higher SBP response to acute stress in males may have evolutionary significance. For example, (Taylor et al., 2000) propose that men typically exhibit a fight or flight stress response whilst women exhibit a 'tend or befriend' response. The stereotypical male stress response, characterised by higher cortisol and sympathetic activation, prepares the organism to mount a physical response to threat. Sex differences across HR and DBP response are less consistently reported (Kelly, Tyrka, Anderson, Price, & Carpenter, 2008; Kirschbaum et al., 1999).

4.5.3 Subjective Stress Response

The ratings of subjective stress and arousal, and mood suggest that participation in a performance audition was sufficient to impinge upon subjective states. The recovery profile demonstrated in subjective measures taken at + 20 and + 30 minutes post-stress exposure suggest the peak feelings of stress, arousal, tension-anxiety, confusion-bewilderment, and overall mood (TMD) were temporally related to exposure to the performance stressor (- 10 and + 10 minutes relative to audition onset). The subjective response profiles elicited by the audition were different to the response pattern typically provoked by laboratory stressors. Subjective responses to laboratory stress protocols typically peak mid- or post-stress onset (Hellhammer & Schubert, 2012). The pronounced anticipatory subjective response shown here may be due to the exposure and anticipated outcome of the audition being more relevant to personal identity. Experience of being exposed to this context before is also likely to underpin the anticipatory response.

4.5.4 Potential Predictors of Cortisol Responsivity

The exploratory analysis of potential predictors of cortisol response reported here suggests that perfectionism may be a promising trait in the identification of a sample of responsive participants. This finding supports that of Wirtz et al. (2007) who demonstrated perfectionism to moderate neuroendocrine stress response to the TSST in males by predicting higher salivary cortisol activation (N = 50). Perfectionism and PASA secondary appraisal were revealed to account for 38% of the adjusted variance in AUCi. Perfectionism alone accounted for 18% of AUCi variance. However, Wirtz et al. identified Concern over Mistakes as the dimension of perfectionism associated with increased cortisol responsivity. Perfectionism: Concern Over Mistakes was considered to be more representative of the classic multidimensional theory of perfectionism. That is, the combination of excessively high standards for performance and overly critical self-evaluation (Frost et al., 1990; Stober, 1998). This pattern of cognition has been associated with negative outcomes such as psychological distress (Antony, Purdon, Huta, & Swinson, 1998), suicidality (O'Connor, 2007), depression and negative affect (Frost et al., 1990), competition anxiety in athletes (Frost, Heimberg, Holt, Mattia, & Neubauer, 1993; Frost & Henderson, 1991), and evaluation anxiety in students (Frost & Marten, 1990). Contrastingly, Perfectionism: Organisation and Personal Standards have been associated with more positive, adaptive outcomes such as goal commitment (Flett, Sawatzky, & Hewitt, 1995), scholastic achievement (Brown et al.,

1999), and positive affect (Frost et al., 1993; Frost et al., 1990). Indeed, Frost et al. (1993) suggested Personal Standards and Organisation may constitute "positive striving" characteristics of perfectionism. It is perhaps surprising then that Perfectionism: Organisation, associated with an over-emphasis on order and efficiency, emerged as the only predictor or stress responsivity in the present study. However, the notion that distinct dimensions of perfectionism influence behavioural and psychological outcomes in consistent adaptive or maladaptive ways is open to debate (Flett & Hewitt, 2006; Miquelon, Vallerand, Grouzet, & Cardinal, 2005; O'Connor, O'Connor, & Marshall, 2007). It is worth noting none of the above studies reporting associations between Perfectionism: Organisation and positive outcomes assessed neuroendocrine parameters, and studies showing positive outcomes did not examine responses under conditions of stress. Furthermore, this perfectionism dimension has been associated with reduced academic performance likely mediated by increased anxiety related to test performance (Seipel & Apigian, 2005).

Wirtz et al. (2007) reported PASA secondary appraisal as a significant predictor of cortisol response. This finding was not replicated here. However, the difference in the sample sizes and composition of both studies is acknowledged. No associations were revealed between cortisol response and other trait factors employed. The small sample size precludes any interpretation of results beyond early explorative analysis. Hence these measures were collected across Studies 2 and 3 reported in this thesis in order to further assess the reliability and generalisability of these measures as predictors of cortisol response.

As expected, the degree to which participants were concerned about conditions of social-evaluative threat (negative judgment by others) was positively related to the corresponding cortisol response. Not all stress contexts, induced in the laboratory or faced in the real-world, are sufficient to provoke a cortisol response. However, the present study supports the findings of Rohleder et al. (2007) by identifying a real-world competitive performance context, characterized by threats to the social-self, capable of eliciting a significant cortisol response. An analogous relationship between perceived social-evaluative threat and cortisol responsivity was also revealed. Rohleder et al. (2007) reported correlations between peak cortisol response and subjective stress relating to the competition judges, whilst a significant relationship between the performers' cortisol response and level of concern at being negatively judged was demonstrated here. This finding emphasises the need to identify a suitable stress context that is characterised by an element of social-evaluative threat.

Correlations between perceived social-evaluative threat prior to audition exposure and cortisol responsivity were greater than those observed between momentary ratings of subjective stress (SACL) across audition exposure and cortisol response. Previous research has suggested that the social-evaluative threat associated with a psychosocial stressor may be more predictive of engendered cortisol response than the subjective stress or anxiety associated with the experience (Gruenewald et al., 2004). Coherence between physiological and subjective stress response is often assumed, largely due to the links between HPA axis activity and key cortical and limbic structures that mediate subjective stress responses (Schlotz et al., 2008). However, empirical evidence of psychophysiological synchrony in acute stress responses is sparse (Mauss, Levenson, McCarter, Wilhelm, & Gross, 2005). Indeed, a recent review of the correspondence between subjective/emotional and physiological (cortisol and cardiovascular) stress responses to TSST studies reported weak and inconsistent correlations (Campbell & Ehlert, 2012). The imperfect coupling of subjective and physiological stress responses and differences between the specific dynamics of the response systems (i.e., rapid subjective and sympathetic response and delayed cortisol response) are often cited to account for this desynchrony (Schlotz et al., 2008). More recent evidence showing that subjective stress ratings during stress exposure (rather than pre and post) are more closely associated with cortisol responsivity (Hellhammer & Schubert, 2012) suggests further work is required to fully understand these dynamics.

4.6 Interim Summary

The study reported in this chapter indicates that a performing arts audition offers a promising naturalistic stress context in which to explore the effects of stress on cognitive performance and the potential for a dietary intervention to modulate this relationship. This context proved sufficient to elicit significant endocrine, cardiovascular and subjective stress responses. Furthermore, the relatively standardised nature of the audition context allows for the collection of regular measures of response parameters. This context also offers the opportunity to undertake an intervention study in a naturalistic environment characterised by genuine social-evaluative threat closely aligned to self-identity is also of interest. The evidence for potential trait predictors of cortisol responsivity seen in Study 1 was examined further in the subsequent studies presented in this thesis.

Chapter 5:
Study 2

Chapter 5 Study 2 - Stress Response and Cognitive Performance under Conditions of a Naturalistic Performance Audition Context Characterised by Social-evaluative Threat

5.1 Introduction

Study 1 demonstrated that involvement in a competitive performance activity was sufficient to provoke significant cortisol, cardiovascular and subjective stress responses. The study described in this chapter provides further piloting of this context to establish its reliability to elicit significant stress responses. In addition, this study was designed to assess parameters of cognitive performance under audition stress and non-stress conditions.

5.1.1 Identification of a Naturalistic Social-evaluative Stress Context

Following the difficulty assessing the level of previous audition experience in Study 1, the examination of cortisol responses across multiple audition exposures to characterise habituation patterns was planned for Study 2. Performance arts students at the University of Leeds were approached to participate in the study. Participants in Study 1 were recruited from the local amateur performing arts community. The opportunity to audition for amateur performance roles in local theatre productions tends to be relatively irregular and auditions for productions can be separated by 6 months. The amateur status of performers also results in availability to participate in auditions for new roles often being limited due to work commitments. Such factors would have resulted in difficulties assessing stress responses in the same sample over repeated auditions. In contrast, performing arts students are required to take part in comparatively regular, standardised auditions as part of their degree course. Involvement in such performance productions is assessed towards final degree classification so likely to represent a significant challenge to participants. Therefore, recruitment from this population offered the opportunity to pilot the audition context over repeated exposures.

5.1.2 Identification of Cognitive Tests

Examination of DM under conditions of stress has predominated in the literature. Stress can have an enhancing or impairing effect upon performance dependent upon the DM process temporally associated with the stress response. However, distinguishing between the DM processes that are being affected by stress induction is a difficult endeavour (Het et al., 2005; Schwabe et al., 2012). As previously discussed in Chapter 2, tests of WM performance may be more reliably sensitive to the impairing effects of stress (Elzinga & Roelofs, 2005; Hsu, Garside, Massey, & McAllister-Williams, 2003; Luethi, Meier, & Sandi, 2009; Schoofs et al., 2009; Young, Sahakian, Robbins, & Cowen, 1999). This impairment is assumed to be the result of prioritising cognitive functions more closely related to the immediate threat posed by a stressor (e.g., DM and attention). Tests of WM that place higher demands upon cognitive resources via engaging multiple components of WM (e.g., manipulation and updating) may be particularly sensitive (Elzinga & Roelofs, 2005). Hence, two WM tests that induce a high level of cognitive demand were selected for piloting in this study – the n-back and ospan tests. Performance on the n-back (Qin, Hermans, Van Marle, Luo, & Fernandez, 2009; Schoofs et al., 2008) and ospan (Schoofs et al., 2009) has also been previously shown to be impaired by acute stress. The n-back and ospan tests require updating and/or active manipulation of information retained in WM (Fletcher & Henson, 2001; Schoofs et al., 2009). Compared to WM tasks that require only passive maintenance of information, such as the digit span forward task, the n-back and ospan require continuous updating and monitoring of new incoming information (see General Methodologies Sections 3.5.3.1 and 3.5.3.2 for more information on these specific WM tests). Briefly, ospan tasks require concurrent online processing of information (completion of mental arithmetic problems) and maintenance of to-be-remembered words for serial recall. This test places high demands on both processing and storage WM functions. The n-back requires continuous monitoring, manipulation and updating of incoming information to identify target stimuli. This test requires active maintenance and dynamic rehearsal of information. The n-back also assesses RT for identification of target and non-target stimuli. Evidence suggests that RT may be particularly sensitive to stress (Schoofs et al., 2008).

5.2 Study Objectives and Hypotheses

Study 2 further explored the feasibility of utilising the performance audition context as a naturalistic acute stressor in future intervention study designs. Exposure to the audition context was expected to induce significant elevations in salivary cortisol, cardiovascular, and subjective stress responses above baseline levels. The recruitment of performing arts students was undertaken to permit the examination of participants' responses over two audition stress exposures. This would be used to assess whether this context was suitable for a repeated measures design by establishing whether patterns of habituation in stress responses were evident. The identification of tests of cognitive performance sensitive to the effects of stress to be employed in the planned nutritional intervention study was identified as a key objective of the thesis. To this end, two tests of WM performance previously shown to be sensitive to pharmacological and psychosocial stress were piloted here. Working memory performance was expected to be impaired under conditions of stress.

The selection of a sample with a response tendency toward high cortisol responsivity was also identified as a key aspect of the planned nutritional intervention study. Considering preliminary evidence of a relationship between trait perfectionism and cortisol responsivity revealed in Study 1, the FMPS was employed again here. The trait measures collected during Study 1 were also collected in the present study to explore whether relationships emerged when assessed with larger combined samples (i.e., combined data from Studies 1 and 2). Additionally, measures of trait anxiety, hostility and alexithymia were collected for exploratory analysis.

5.3 Methods

5.3.1 Sample

Seventeen (12 female; 5 male) University of Leeds performing arts students undertaking performance auditions participated (see Table 5.1 for sample characteristics). Participation in auditions formed part of the students' degree programme and was assessed as part of their final degree classification. Successful auditionees would perform in theatre productions held at the university. No monetary reward was given for successful auditions. Study eligibility was determined from screening questionnaires completed at an audition rehearsal meeting. Ten of 12

female participants were taking OC's; two participants not taking OC's were in the follicular phase of their menstrual cycle at audition.

5.3.2 Inclusion and Exclusion Criteria

Participants were recruited using the following criteria.

5.3.2.1 Inclusion criteria

- Male or female over 18 years of age

5.3.2.2 Exclusion criteria

As reported in Methodologies Section3.3.4.

5.3.3 Design

The study conformed to a naturalistic repeated measures design assessing endocrine, cardiovascular and subjective responses of individuals on a non-audition day (to establish baseline values), and whilst undertaking performing arts auditions. Cognitive performance on two tests of working memory was also assessed on a non-audition day and post-audition stress exposure.

As discussed in General Methodologies Section 3.5.1 multiple exposures to cognitive tests to establish a baseline were implemented to reduce potential practice effects. To this end, participants attended a short non-audition day visit at the Institute of Psychological Sciences. Participants completed each cognitive test three times. The third exposure was subsequently used as a baseline performance measure. Cardiovascular (BP) and subjective measures (SACL and POMS) were also collected to establish baseline measures temporally removed from audition stress exposure. These measures also provided confirmation that baseline cognitive performance was undertaken under non-stress conditions.

5.3.4 Endocrine Measures

5.3.4.1 Cortisol

Salivary cortisol was collected on the audition day – 20, – 10, + 10, + 20, and + 30 minutes relative to audition onset. Salivary-free cortisol concentrations were determined using a Salivary Cortisol Enzyme Immunoassay kit (EIA; Sarstedt;

Nümbrecht, Germany; described in Section 3.2.1.1). Intra- and inter-assay variability was below 8.2 and 11.4% respectively.

5.3.5 Physiological Measures

5.3.5.1 Blood pressure

An Omron M7 ambulatory blood pressure monitor was used to measure SBP and DBP. Three baseline blood pressure measures were collected on a non-audition day 1-2 days prior to the audition. Blood pressure was also measured on the audition day at – 20, – 10, + 10, + 20 and + 30 minutes relative to audition onset.

5.3.6 Screening Measures

5.3.6.1 Hospital Anxiety and Depression Scale (HADS)

Participants completed the HADS 5-7 days prior to the audition day (General Methodologies Section 3.3.2).

5.3.7 Subjective Measures

The following measures were employed to assess baseline chronic stress level and acute subjective responses to the audition. More detailed descriptions of measures are given in the General Methodologies Section where indicated.

5.3.7.1 Perceived Stress Scale (PSS)

Participants completed the PSS 5-7 days prior to the audition day (General Methodologies Section 3.3.1).

5.3.7.2 Social-evaluative threat visual analogue scales (VAS)

As a measure of subjective social-evaluative threat participants were asked to indicate how stressful/threatening they found aspects of the audition process using 100 mm VAS anchored by the descriptors "*totally disagree"* to "*totally agree"* (Appendix 18). The original seven statements relating to elements of social-evaluative threat used in Study 1 were again employed. An additional four statements relating to general feelings of stress unrelated to social-evaluative threat were added (e.g., general health and getting to the venue on time). The non-audition related stress factors were added to assess if stress appraisals specific to the social-evaluative context, or feelings of

stress in general, were related to cortisol response. The social-evaluative threat VAS were completed – 10 minutes prior to the audition.

5.3.7.3 Stress and Arousal Checklist (SACL)

Three baseline SACL measures were collected on a non-audition day 1-2 days prior to the audition. The SACL was completed on the audition day – 20, – 10, + 10, + 20 and + 30 minutes relative to audition onset (General Methodologies Section 3.2.3.1).

5.3.7.4 Profile of Mood States – short form (POMS-SF)

The short form of the POMS was employed as participants in Study 1 indicated the full version induced fatigue (although this was not evident from responses on the POMS Fatigue-inertia subscale; see Section 4.4.4.2). Three baseline POMS-SF measures were collected on a non-audition day 1-2 days prior to the audition. The POMS-SF was completed on the audition day – 20, – 10, + 10, + 20 and + 30 minutes relative to audition onset (General Methodologies Section 3.2.3.3).

5.3.7.5 Primary Appraisal Secondary Appraisal (PASA)

The PASA was completed – 20 minutes relative to audition onset (General Methodologies Section 3.2.3.4).

5.3.8 Trait Personality Measures

The following trait personality measures were employed in order to explore potential trait predictors of acute cortisol responsivity to stress. More detailed descriptions of all personality measures are given in the General Methodologies (sections shown in parentheses). The reliability coefficients for each measure are given here. All personality measures were completed five days prior to auditions.

- Frost Multidimensional Perfectionism Scale (Section 3.4.1). Acceptable reliability coefficients were found for subscales: *Parental Expectation* (r_α = .80), *Organisation* (r_α = .92), *Parental Criticism* (r_α = .80), *Concern over Mistakes* (r_α = .92), *Personal Standards* (r_α = .86), and *Total Perfectionism Score* (r_α = .88). The subscale *Doubts about Actions* was again shown to be less reliable that the other subscales (r_α = .66). Less consistent reliability coefficients have previously been reported for this subscale in Study 1 (r_α = .59; Section 4.3.8) and the literature (e.g. r_α = .66; Harvey et al., 2004).

- Rotter's external-internal Locus of Control Scale (Section 3.4.2; r_α = .81)
- Rosenberg Self-Esteem Scale (Section 3.4.3; r_α = .74)
- Neuroticism (IPIP-N; Section 3.4.4; r_α = .70)
- Toronto Alexithymia Scale 20 Item Version (TAS-20; Section 3.4.5). Acceptable reliability coefficients were found for the alexithymic subscales of *Difficulty Describing Feelings* (r_α = .84) and *Difficulty Identifying Feelings* (r_α = .86). The subscale *Externally-Orientated Thinking* was found to be less reliable (r_α = .66). Less consistent reliability coefficients (ranging from r_α = .52 – 66) have been previously reported for this alexithymic subscale (Bagby, Parker, & Taylor, 1994; Bressi et al., 1996; Loas et al., 2001). The reliability of this subscale may be particularly vulnerable to inconsistent internal reliability cross-culturally (e.g. Fukunishi, Nakagawa, Nakamura, Kikuchi, & Takubo, 1997).
- State-Trait Anxiety Inventory (STAI; Section 3.4.6; r_α = .84)
- Cook-Medley Hostility Scale (Ho Scale; Section 3.4.7; r_α = .83)
-

5.3.9 Tests of Cognitive Performance

5.3.9.1 2-back

A full description of this test is detailed in the General Methodologies Section 3.5.3.1. Three trial blocks of 50 stimuli were presented to participants. Digits were presented for 500 ms with an inter-stimulus delay of 1000 ms. Participants completed the 2-back test three times on a non-audition day to establish a baseline performance level (with third exposure used as baseline).

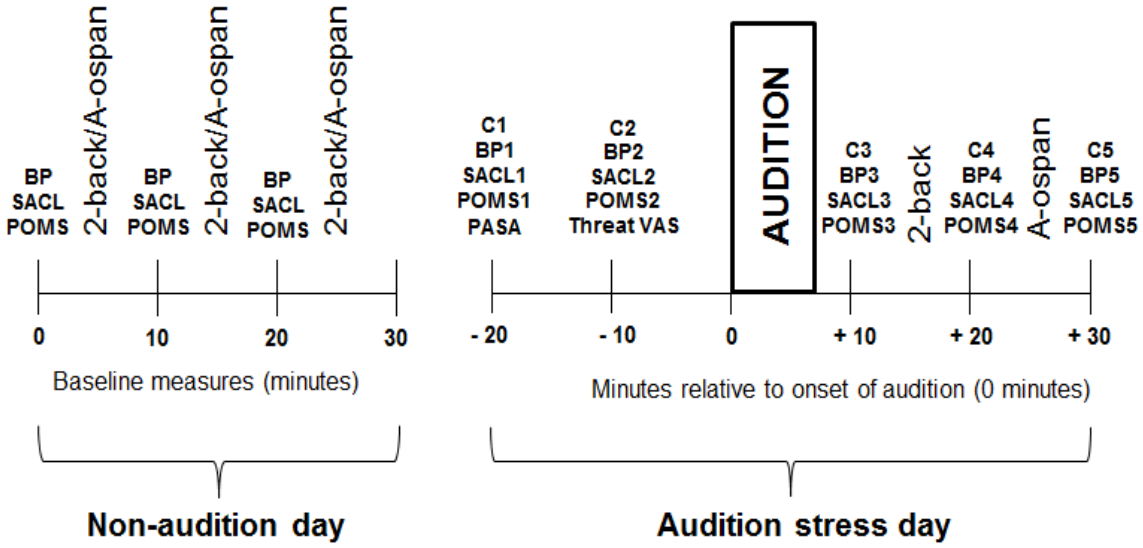
5.3.9.2 A-ospan

A full description of this test is detailed in the General Methodologies Section 3.5.3.2. The version employed here comprised five stimulus blocks of 3 and 4 set size operations (10 operations in total). The maximum score possible for A-ospan score and total was 35. Participants completed the A-ospan three times on a non-audition day to establish a baseline performance level (third exposure was used as baseline).

5.3.10 Procedure

Participants attended an initial non-audition day visit at the Institute of Psychological Sciences to establish baseline measures. The baseline visit was undertaken to expose participants to the cognitive tests and collect measures of blood pressure and subjective states at a time temporally removed from the audition. Baseline visits were completed 1-2 days prior to audition exposure at a time within 2 hr of proposed audition onset time. Measures of blood pressure, subjective stress (SACL), and mood (POMS-SF) were collected at 10 minute intervals: 0, + 10, and + 20 minutes relative to baseline visit commencement. The 2-back and A-ospan were completed on three occasions in between collection of physiological and subjective measures. A procedural timeline of the study is shown in Figure 5.1.

Auditions were undertaken in front of a panel of four evaluative judges in a theatre auditorium setting. The audition judging panel was comprised of three fellow performance degree students undertaking directing and producing roles, and the degree course leader. Each auditionee was required to perform a scripted spoken scene (approximately 8 minutes in duration) in front of the selection panel judges. Testing occurred between 1145 and 1700 hrs. Participants were asked to refrain from exhaustive exercise, consuming meals or caffeinated/low pH drinks, and brushing teeth 1 hour prior to testing. Testing was undertaken in a rehearsal waiting room at the audition venue. Each participant reported to the experimenter 20 minutes prior to being called into the audition to complete pre-audition physiological and subjective measures. A BP monitor was fitted whilst participants completed experimental measures but removed for the audition performance. Upon completion of the audition, participants returned immediately to the rehearsal waiting room to complete post-audition measures and cognitive tests.



C = Salivary cortisol, BP = Blood pressure, Threat VAS = Social-evaluative threat visual analog scale, POMS = Profile of Mood States-short form, SACL = Stress and Arousal Checklist, PASA = Primary Appraisal Secondary Appraisal

Figure 5.1 Procedural timeline

5.3.11 Ethical Approval

All participants provided written informed consent one week prior to study inclusion. The experimenter presented the study and disseminated participant information sheets to potential participants at course lectures. Given the potentially stressful nature of audition participation, the experimenter verbally reiterated the purpose and procedures of study participation to interested performers. The experimenter also liaised closely with the course leader to limit disruption of the audition process. Particular emphasis was given to the participants' right to withdraw from the study at any time before or during study participation. The study was approved by the University of Leeds' Institute of Psychological Sciences Research Ethics Committee (Ref: 11-0146; submitted and approved October 2011). All participants were paid a £10 honorarium on study completion.

5.3.12 Statistical Analysis

Data for cortisol were positively skewed and normalised using a logarithmic transformation. One participant's A-ospan performance data was removed from analysis due to being a significant outlier (> 4 *SD* below sample mean). Independent T-tests were employed to compare participant characteristics across sex.

The SAS-mixed models procedure (PROC MIXED) was employed to examine the within-subjects change in stress response outcome variables across audition exposure. Participant ID was entered as a random factor; time and sex were entered as fixed factors. Age, BMI, PSS score were also initially included as covariates but subsequently removed from models due to non-significance across all measures. The mixed models procedure was employed to analyse the within-subjects change in cognitive performance across baseline and post-audition performance with sex and time entered as fixed factors. No effect of sex was revealed so cognitive performance outcomes were compared using paired T-tests.

Partial correlations (two-tailed) were employed to explore the relationship between social-evaluative threat VAS ratings and cortisol responses, and subjective stress ratings (SACL) and cortisol and cardiovascular responses. The statistical approach employed for exploratory regression analyses is described in General Methodologies Section 3.6.1). The method of selecting covariance structure for the mixed models is described in General Methodologies Section 3.6.2.

5.4 Results

5.4.1 Participant Characteristics

Independent T-tests revealed no significant differences between males and females in terms of age, $t(15) = 0.49$, $p = .63$, BMI, $t(15) = -0.96$, $p = .35$, PSS score, $t(15) = 0.13$, $p = .90$, HADS-A, $t(15) = -0.57$, $p = .58$, or HADS-D, $t(15) = -1.78$, $p = .11$. Participant characteristics are shown in Table 5.1.

Table 5.1 Participant characteristics by sex (*N* = 17)

	Males		Females	
	$\overline{X}$ (SEM)		$\overline{X}$ (SEM)	
Age	20.40	(1.82)	20.75	(1.14)
BMI (kg/m²)	22.68	(1.19)	21.94	(1.54)
PSS	15.20	(3.35)	15.73	(4.63)
HADS-D	5.20	(1.30)	3.92	(1.38)
HADS-A	4.00	(0.71)	3.42	(2.19)

PSS - Perceived Stress Scale (score range 0-40).
HADS - Hospital Anxiety and Depression Scale (score range 0 - 21 for depression and anxiety scales).

5.4.2 Salivary Cortisol Response

The audition stressor had no significant effect on cortisol response across audition exposure reflected in a non-significant main effect of time, $F(4,58) = 0.48$, $p = .75$ (Figure 5.2). The mean salivary cortisol level increase from baseline (– 20 minutes = 4.80 nmol/L) to peak (+ 30 minutes = 4.90 nmol/L) following audition exposure was 0.10 nmol/L, representing a nominal increase of 2%. No significant effect of sex was revealed, $F(1,15) = 1.94$, $p = .18$. However, Figure 5.2 indicates a tendency for greater mean cortisol levels in female participants across the audition. Male participants demonstrated a small, non-significant increase in cortisol pre-audition onset but levels had returned to baseline levels post-audition.

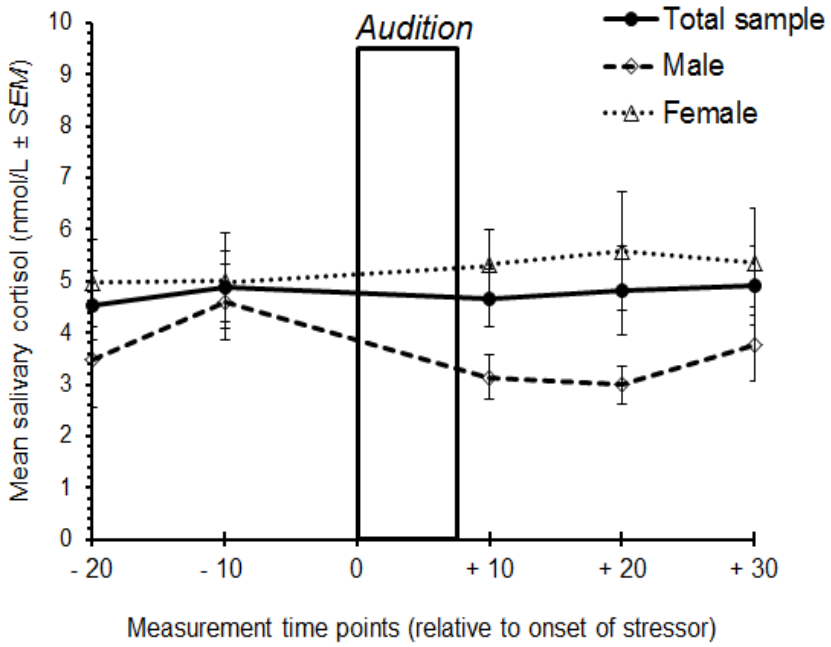


Figure 5.2 Mean (± *SEM)* salivary cortisol response (nmol/L) pre + post audition in the total sample and by sex

5.4.3 Cardiovascular Response

5.4.3.1 Systolic blood pressure

Exposure to audition stress provoked significant changes in SBP reflected in a main effect of time, $F(7,103) = 15.81$, $p < .001$ (Figure 5.3). A significant main effect of sex,

$F(1,15) = 9.50$, $p < .001$, was also revealed for SBP responses. The time×sex interaction was however, non-significant, $F(7,103) = 0.87$, $p < .53$.

In the whole sample, SBP was significantly higher at – 20, – 10, + 10, + 20, and + 30 minutes relative to onset of audition exposure compared to all three baseline measures (all significant at $p < .04$). Systolic BP was also significantly higher at – 20, – 10, and + 10 compared to measures at + 20 and + 30 minutes (all significant at $p < .03$). Significant elevations within the SBP response profile were demonstrated by male and female participants. However, male participants demonstrated significantly higher SBP responses across all sampling time points (all significant at $p < .03$).

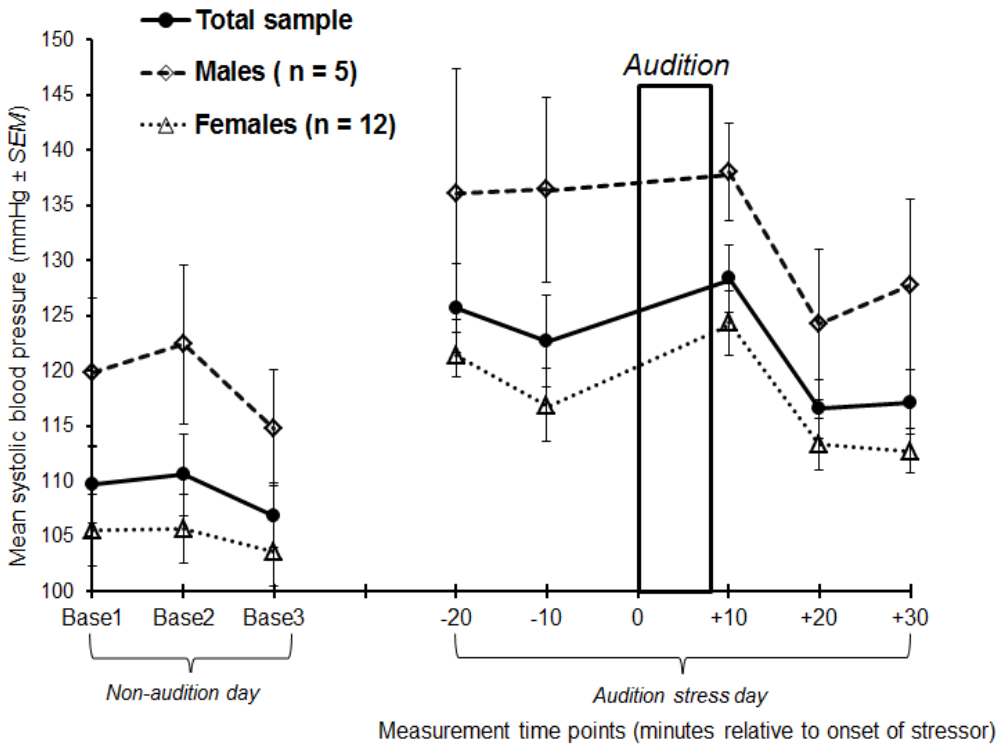


Figure 5.3 Mean (± *SEM)* SBP response (mmHg) pre + post audition exposure in the whole sample and by sex

5.4.3.2 Diastolic blood pressure

Exposure to audition stress provoked significant changes in DBP reflected in a main effect of time, $F(7,103) = 9.35$, $p < .001$ (Figure 5.4). Mean DBP was significantly higher – 20, – 10, + 10, + 20, and + 30 minutes relative to the onset of audition exposure compared to all three baseline measures (all significant at $p < .03$). No

significant differences between measurement time points on the audition day were revealed.

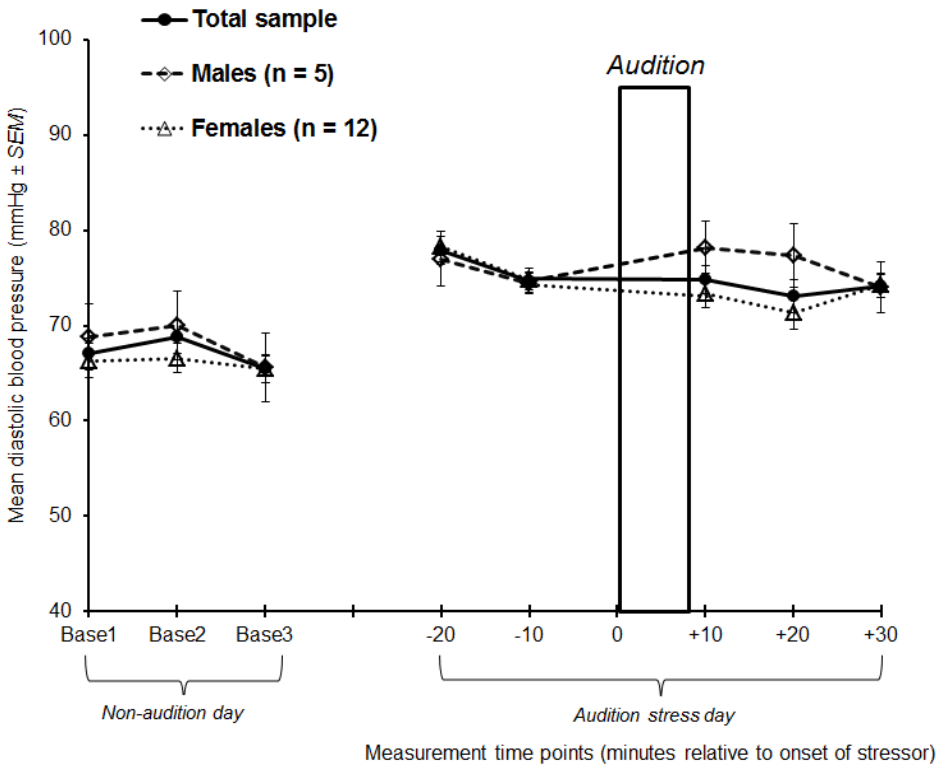


Figure 5.4 Mean (± *SEM)* DBP response (mmHg) pre + post audition exposure in the whole sample and by sex

5.4.4 Subjective Response

5.4.4.1 Stress and Arousal Checklist

Audition stress exposure had a significant effect on subjective stress reflected in a main effect of time for SACL stress ratings, $F(7,103) = 6.18$, $p < .001$. Audition stress exposure did not significantly affect subjective arousal ratings, $F(7,103) = 1.73$, $p = .10$. The means (± *SEM*) of subjective stress and arousal are shown in Table 5.2. Subjective stress ratings were significantly lower at Baseline 1 compared to all other measurement time points (all significant at $p < .01$). Participants reported feeling significantly higher levels of subjective stress at – 20, – 10, and + 10 minutes compared to Baselines 2 and 3 (all significant at p < .01). A decline in subjective stress rating was evident after 30 minutes with significantly lower ratings at + 30 compared to – 20, – 10 and + 10 minutes (all significant at $p < .04$).

5.4.4.2 Profile of Mood States

Audition stress exposure had a significant effect on subjective mood ratings reflected in main effects of time for Tension-anxiety, $F(7,103) = 4.73$, $p < .001$, Vigour-activity, $F(7,103) = 3.59$, $p < .001$, and Fatigue-inertia, $F(7,103) = 2.21$, $p = .04$. Non-significant effects of audition exposure were revealed for ratings of Depression-dejection, $F(7,103) = 0.37$, $p = .92$, Anger-hostility, $F(7,103) = 0.85$, $p = .55$, Confusion-bewilderment, $F(7,103) = 0.33$, $p = .94$, and TMD, $F(7,103) = 0.65$, $p = .70$.

All mean ratings (± *SEM*) are shown in Table 5.2. Subjective Tension-anxiety ratings were significantly higher than all baseline measures at – 20, – 10, and + 10 minutes relative to audition onset (all significant at $p < .003$). Some recovery towards baseline rating of subjective Tension-anxiety was evident after 30 minutes with significantly lower ratings at + 30 compared to – 20, – 10, and + 10 minutes (all significant at $p < .02$).

Heightened subjective arousal was evident at baseline with ratings of Vigour-activity at Baseline 1 significantly higher than Baseline 3, and + 20 and + 30 minutes post-audition onset (all significant at $p < .05$). Ratings at Baseline 2 were also significantly higher than + 30 minutes post-audition ($p < .001$). The peak mean rating of Vigour-activity at + 10 was significantly higher than Baseline 3, and + 20 and + 30 minutes post-audition (all significant at $p < .03$). A decline in Vigour-activity rating was evident after 30 minutes with significantly lower ratings at + 30 compared to – 20, – 10 and + 10 minutes (all significant at $p < .03$).

Participants reporting significantly higher ratings of Fatigue-inertia + 10 minutes post-audition than Baseline 2 and 3, and – 20 and – 10 minutes pre-audition (all significant at $p < .01$). Greater fatigue was also reported at + 20 compared to Baseline 3, and + 30 compared to – 10 minutes pre-audition (both significant at $p < .01$).

Table 5.2 Mean (± *SEM)* subjective stress/arousal (SACL) and mood (POMS) responses across baseline and audition exposure. Score ranges are shown in parentheses next to each measure dimension

	Baseline measures						Audition stress measures									
							Time in minutes relative to audition onset									
	Baseline 1		Baseline 2		Baseline 3		- 20		- 10		+ 10		+ 20		+ 30	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
SACL																
Stress (18 - 72)	**29.59**	1.70	**35.71**	2.50	**36.59**	2.60	**45.63**	2.70	**48.44**	2.90	**44.00**	2.80	**40.18**	2.50	**37.41**	1.60
Arousal (12 - 48)	**33.77**	1.60	**34.88**	1.90	**31.77**	2.40	**35.69**	1.90	**35.06**	1.80	**37.06**	1.70	**33.82**	1.70	**30.06**	1.80
POMS																
Tension-anxiety (0 - 24)	**3.18**	0.70	**3.35**	0.90	**3.12**	0.60	**8.75**	1.40	**9.81**	1.70	**6.76**	1.40	**5.25**	1.20	**3.76**	1.20
Depression-dejection (0 - 32)	**4.25**	0.70	**2.65**	0.20	**3.41**	1.00	**4.63**	1.50	**4.13**	1.70	**4.35**	1.10	**3.12**	0.90	**3.52**	1.00
Anger-hostility (0 - 28)	**1.35**	0.80	**1.12**	0.50	**2.94**	1.50	**1.13**	0.70	**0.75**	0.70	**1.12**	0.40	**0.35**	0.10	**0.71**	0.30
Vigour-activity (0 - 24)	**8.59**	1.10	**8.35**	1.40	**6.24**	1.40	**8.94**	1.40	**8.69**	1.30	**9.53**	1.10	**6.76**	1.10	**4.71**	1.00
Fatigue-inertia (0 - 20)	**3.82**	0.90	**3.06**	0.90	**3.47**	1.00	**3.69**	1.10	**2.94**	1.10	**4.65**	0.90	**4.12**	0.70	**4.65**	1.10
Confusion-bewilderment (0 - 20)	**2.06**	0.50	**2.59**	0.40	**1.94**	0.40	**3.31**	1.20	**3.38**	1.20	**3.00**	0.90	**2.59**	0.80	**2.71**	1.00
TMD (0 - 124)	**14.65**	2.25	**12.77**	1.80	**14.88**	3.25	**20.24**	4.91	**19.77**	5.25	**19.88**	3.75	**15.41**	2.89	**15.35**	3.73

SACL = Stress and Arousal Checklist; POMS = Profile of Mood States; TMD = Total Mood Disturbance

Highlighted sections denote significant differences across the response profile

5.4.5 Cognitive Performance

5.4.5.1 2-back

Paired T-tests revealed a significant effect of performance context on working memory parameters for both 2-back target accuracy, $t(16) = 2.74$, $p = .01$ (Figure 5.5), and total accuracy, $t(16) = 3.10$, $p < .001$ (Figure 5.6). Target and total accuracy were significantly lower post-audition exposure compared to baseline. Audition exposure had no effects on measures of RT (target RT, $t(16) = 0.81$, $p = .43$; non-target RT, $t(16) = - 0.03$, $p = .98$).

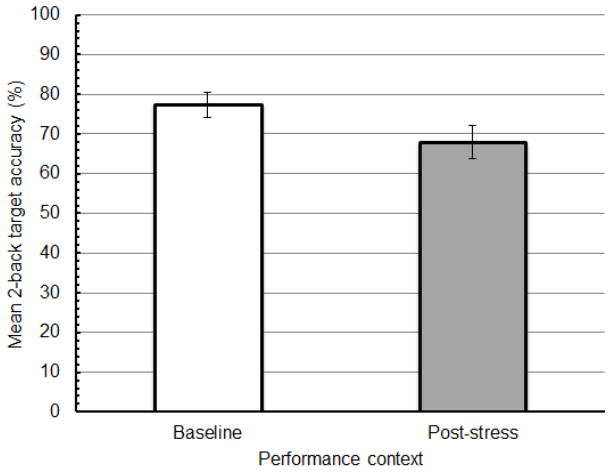


Figure 5.5 Mean (± *SEM*) 2-back target accuracy performance across baseline and post-audition stress

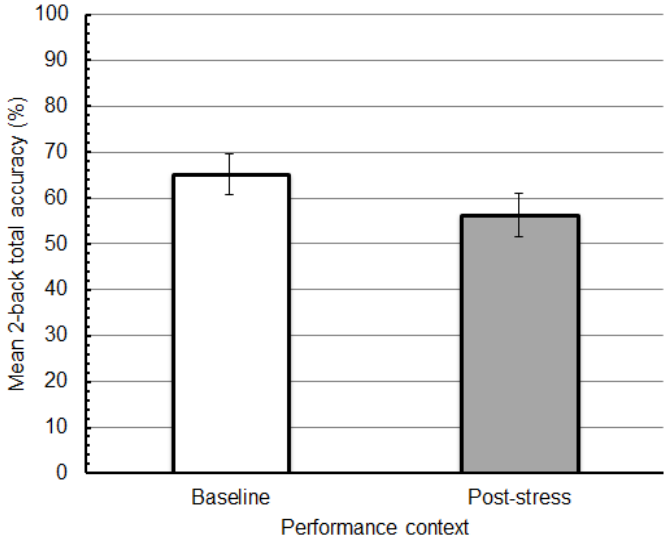


Figure 5.6 Mean (± *SEM*) 2-back total accuracy performance across baseline and post-audition stress

5.4.5.2 A-ospan

Paired T-tests revealed a significant effect of performance context on WM parameters for A-ospan total, $t(15) = 2.12$, $p = .04$ (Figure 5.7). A-ospan total was significantly lower post-audition exposure compared to baseline. Audition exposure had no significant effects on A-ospan score, $t(15) = 1.85$, $p = .08$, accuracy errors, $t(15) = -1.13$, $p = .28$, or speed errors, $t(15) = 1.16$, $p = .26$.

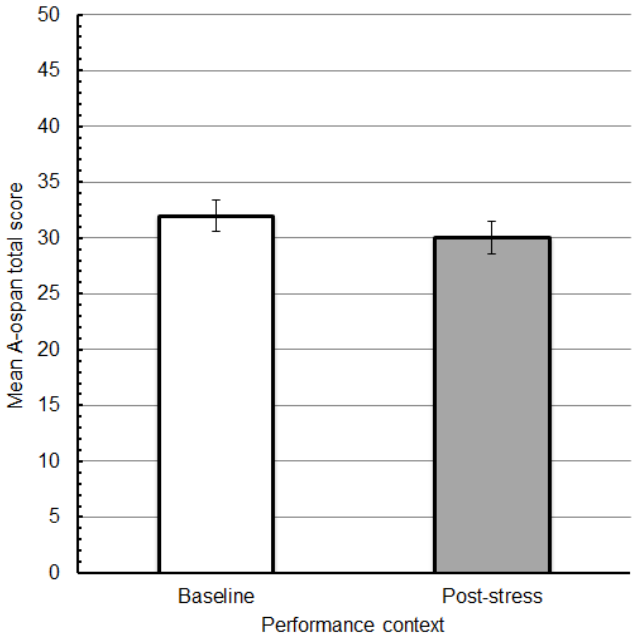


Figure 5.7 Mean (± *SEM*) A-ospan total across baseline and post-audition stress

5.4.6 Predictors of Cortisol Responsivity

5.4.6.1 Associations between trait variables and cortisol response

Partial correlations (two-tailed) controlling for sex and age revealed significant relationships between Perfectionism: Doubts about Actions and salivary cortisol level at – 20 minutes pre-audition onset, $r(13) = .60$, $p = .02$, and AUCg, $r(13) = .61$, $p = .02$. Exploratory regression analysis (controlling for age, sex, and BMI) produced non-significant models for this dimension of perfectionism when cortisol levels at – 20 minutes and AUCg were entered as outcome variables (see Table 5.3 for regression model values). Considering the lack of significant excursion from baseline cortisol

levels across audition exposure in this sample (AUCi, $\overline{X}$ = 19.10 ± 2.20 nmol/L; AUCg, $\overline{X}$ = 0.95 ± 2.25; delta increase, $\overline{X}$ = - 1.43 ± 0.89), the correlation between Perfectionism: Doubts about Actions and cortisol is likely to be representative of a small subset of the sample which is evident when observing the scatterplots (shown in Appendix 19).

Table 5.3 Hierarchical multiple regression analyses of relationships between Perfectionism: Doubts about Actions and salivary cortisol AUCg and – 20 minutes (controlling for sex, age, and BMI)

						Measurement time point relative to audition onset				
		AUCg				- 20				
		B	*SE B*	*β*	*F* change		*B*	*SE B*	*β*	*F* change
Step 1										
	Constant	5.44	2.06			Constant	2.75	0.93		
	Sex	0.03	0.22	.03		Sex	-0.09	0.09	-.21	
	Age	-0.02	0.08	-.06		Age	-0.07	0.03	-.48	
	BMI	-0.2	0.07	-.62	2.68	BMI	-0.03	0.03	-.21	2.25
Step 2										
	Constant	5.97	2.10			Constant	2.45	0.92		
	Sex	0.00	0.22	-.003		Sex	-0.07	0.09	-.16	
	Age	-0.04	0.08	-.11		Age	-0.06	0.03	.03	
	BMI	-0.17	0.07	-.56		BMI	-0.04	0.03	-.28	
	Perfectionism: DA	0.04	0.04	.25	1.22	Perfectionism: DA	0.22	0.02	.32	2.14

AUCg Step 1: R^2 = .38, ΔR^2 = .24, $F(3,16)$ = 2.68, p = .09; Step 2: R^2 = .44, ΔR^2 = .25, $F(4,16)$ = 2.35, p = .11.
- 20 minutes, *Step 1:* R^2 = .34, ΔR^2 = -.19, $F(3,16)$ = 2.25, p = 13.; Step 2: R^2 = .44, ΔR^2 = .25, $F(4,16)$ = 2.37, p = .11.
Perfectionism: DA - Perfectionism Doubts about Actions

The cortisol and trait personality data from Studies 1 and 2 were combined, and partial (two-tailed) correlation analyses were repeated to ascertain if any relationships emerged when the data were aggregated and the sample size increased. Salivary cortisol AUCg and delta increase were positively skewed for this combined data set and normalised using logarithmic transformations. Controlling for sex and age, partial correlations revealed significant relationships between Perfectionism: Personal Standards and AUCg, $r(29)$ = .42, p = .02, and cortisol levels at + 20 minutes, $r(29)$ = .46, p = .008. Significant relationships between Perfectionism: Organisation and AUCg, $r(29)$ = .53, p = .002, AUCi, $r(29)$ = .41, p = .02, delta increase, $r(29)$ = .45, p = .01, and cortisol levels at + 10, $r(29)$ = .52, p = .002, + 20, $r(29)$ = .54, p = .002, and + 30, $r(29)$ = .46, p = .01, minutes were also revealed.

Exploratory regression analysis revealed the control variables (age, sex, and BMI) did not significantly predict AUCg, AUCi, or delta increase (step one). The inclusion of Perfectionism: Organisation and Perfectionism: Personal Standards at step two

resulted in models that accounted for 38% of the adjusted variance in salivary cortisol AUCg and 25% of the adjusted variance in delta increase. Perfectionism: Organisation emerged as the only significant predictor in the models. A non-significant model was revealed for AUCi (p = .09 [see Table 5.4 for regression model values for aggregated cortisol measures]).

Analysis revealed the control variables (age, sex, and BMI) did not significantly predict cortisol levels at + 10 and + 20 minutes post audition onset (step one). The inclusion of Perfectionism: Organisation and Perfectionism: Personal Standards at step two resulted in models that accounted for 29% of the adjusted variance in salivary cortisol at + 10 minutes and 37% of the adjusted variance in cortisol at + 20 minutes. Perfectionism: Organisation emerged as the only significant predictor of cortisol levels at both time points (see Table 5.5 + 10 and + 20 minutes for regression model values).

Age emerged as a significant predictor of salivary cortisol levels in the first step of the regression model predicting cortisol levels at + 30 minutes post audition onset which accounted for 22% of the variance. The inclusion of Perfectionism: Organisation and Perfectionism: Personal Standards at step two significantly increased the adjusted variance accounted for by 11% over and above the control variables (age, sex, and BMI). Age, Sex and Perfectionism: Organisation emerged as significant predictors in the final model (see Table 5.5 + 30 minutes for regression model values).

Table 5.4 Hierarchical multiple regression analyses of relationships between Perfectionism: Organisation and Personal Standards, and aggregated measures of salivary cortisol (controlling for sex, age, and BMI).

		AUCg					***AUCi***					***Delta Increase***			
		B	*SE B*	*β*	*F* change		*B*	*SE B*	*β*	*F* change		*B*	*SE B*	*β*	*F* change
Step 1															
	Constant	0.37	0.47			Constant	1.78	32.13			Constant	-0.31	0.63		
	Sex	0.12	0.08	.24		Sex	0.65	5.59	.02		Sex	0.58	0.11	.09	
	Age	0.03	0.01	.20		Age	1.11	0.66	.31		Age	0.28	0.13	.27	
	BMI	0.01	0.02	.10	2.81	BMI	-0.68	1.34	-.01	0.93	BMI	0.02	0.03	.13	2.25
Step 2															
	Constant	0.60	0.42			Constant	15.73	29.94			Constant	-0.27	0.59		
	Sex	0.17	0.07	.23		Sex	3.76	5.26	.12		Sex	0.12	0.10	.19	
	Age	0.01	0.01	.19		Age	0.47	0.66	.13		Age	0.02	0.01	.25	
	BMI	-0.01	0.10	-.07		BMI	-1.74	1.30	-.24		BMI	0.004	0.03	.03	
	Perfectionism PS	0.01	0.01	.24		Perfectionism PS	0.16	0.53	.06		Perfectionism PS	-0.01	0.01	-.20	
	Perfectionism Org	0.01	0.01	.41***	6.36*	Perfectionism Org	1.01	0.49	.49***	3.75***	Perfectionism Org	0.03	0.01	.58**	3.76***

AUCg Step 1: $R^2 = .23$, $\Delta R^2 = .15$, $F(3,32) = 2.81$, $p = .07$; Step 2: $R^2 = .47$, $\Delta R^2 = .38$, $F(5,32) = 4.85$, $p = .003$.
AUCi Step 1: $R^2 = .08$, $\Delta R^2 = -.01$, $F(3,32) = 0.93$, $p = .44$; Step 2: $R^2 = .29$, $\Delta R^2 = .15$, $F(5,32) = 2.16$, $p = .09$.
Delta Increase: *Step 1:* $R^2 = .19$, $\Delta R^2 = .11$, $F(3,32) = 2.25$, $p = .10$; Step 2: $R^2 = .37$, $\Delta R^2 = .25$, $F(5,32) = 3.11$, $p = .02$.
* $p < .005$, ** $p < .01$, *** $p < .04$
Perfectionism PS - Personal Standards, Perfectionism Org - Organisation

Table 5.5 Hierarchical multiple regression analyses of relationships between Perfectionism: Organisation and Personal Standards, and post-audition salivary cortisol measures (controlling for sex, age, and BMI)

	Measurement time point (relative to audition onset)											
	+ 10				+ 20				+ 30			
	B	*SE B*	*β*	*F* change	*B*	*SE B*	*β*	*F* change	*B*	*SE B*	*β*	*F* change
Step1												
Constant	-7.33	13.01			-6.25	14.47			-15.57	9.66		
Sex	0.44	2.26	.03		1.86	2.51	.13		2.83	1.68	.27	
Age	0.47	0.27	.33		0.69	0.30	.42		0.58	0.20	.48*	
BMI	0.16	0.54	.05	1.28	-0.18	0.61	-.05	1.92	0.21	0.40	.09	4.07**
Step 2												
Constant	-0.82	11.32			1.16	12.18			-11.38	9.09		
Sex	1.89	1.99	.15		3.49	2.14	.24		3.78	1.60	.35***	
Age	0.15	0.25	.10		0.31	0.27	.18		0.41	0.20	.34***	
BMI	-0.37	0.49	-.13		-0.82	0.53	-.24		-0.72	0.39	-.04	
Perfectionism PS	0.17	0.20	.17		0.26	0.21	.23		-0.03	0.16	-0.04	
Perfectionism Org	0.44	0.18	.52**	6.30*	0.48	0.19	.49**	7.61*	0.33	0.14	.47***	4.21*

+ 10 minutes Step 1: $R^2 = .12, \Delta R^2 = .03, F(3,32) = 1.28, p = .30$; Step 2: $R^2 = .40, \Delta R^2 = .29, F(5,32) = 6.30, p = .01$.
+ 20 minutes Step 1: $R^2 = .17, \Delta R^2 = .08, F(3,32) = 1.92, p = .15$; Step 2: $R^2 = .47, \Delta R^2 = .37, F(5,32) = 4.72, p = .003$.
+ 30 minutes Step 1: $R^2 = .29, \Delta R^2 = .22, F(3,32) = 4.07, p = .02$; Step 2: $R^2 = .44, \Delta R^2 = .33, F(5,32) = 4.21, p = .006$.
* $p < .005$, ** $p < .01$, *** $p < .04$
Perfectionism PS - Personal Standards, Perfectionism Org - Organisation
* $p < .007$, ** $p < .02$, *** $p < .05$

5.4.7 Subjective Stress and Physiological Stress Response

Partial correlations (two-tailed) revealed a significant positive relationship between subjective ratings of stress (SACL) and SBP at – 10 minutes, $r(12) = .58$, $p = .03$, prior to audition onset. No other significant relationships were found.

5.4.8 Social-evaluative Threat and Cortisol Response

Partial correlations (two-tailed) controlling for sex and age revealed no significant relationships between social-evaluative threat and general stress VAS and cortisol measures. Independent T-tests were undertaken to explore the possibility that the audition context did not induce as much perceived social-evaluative threat as the audition context in Study 1. In Study 1 auditionees reported descriptively higher perceived fear of being judged negatively by the evaluative panel ($\overline{X} = 52.94 \pm 6.39$) and higher concern over factors outside of their control affecting their performance ($X = 60.25 \pm 6.32$) compared to Study 2 ($\overline{X} = 48.76 \pm 5.05$ and $\overline{X} = 45.30 \pm 8.24$ respectively). However, neither comparison reached statistical significance: fear of being judged, $t(31) = 0.52$, $p = .61$, factors outside control, $t(31) = 1.43$, $p = .16$.

5.5 Discussion

5.5.1 Salivary Cortisol Response

Exposure to the audition stressor failed to perturb the HPA axis and elicit a cortisol response in this sample. Naturalistic stressors have been shown to have different potencies to provoke significant cortisol responses (Biondi and Picardi, 1999; Michaud, et al., 2008). However, this finding is surprising considering that this type of context was previously shown to be sufficient to induce a significant endocrine response in Study 1.

Previous evidence has suggested that level of social-evaluative threat and perception of control may play key roles in the extent to which a cortisol response is elicited (Dickerson & Kemeny, 2004). Study 1, and Rohleder et al. (2007), demonstrated the importance of social-evaluative threat related to being judged by the competition/audition judges in a naturalistic performance context. Manipulation of the level of social-evaluative threat has also been shown to moderate cortisol response in laboratory studies (e.g., Ginis, Strong, Arent, & Bray, 2012; Gruenewald et al., 2004).

The student performance auditions may have been characterised by lower levels of social-evaluative threat. However, only descriptively lower levels of social-evaluative threat relating to the audition judges and higher perceived controllability were reported by participants in this study compared to Study 1. Participants in Study 1 auditioned to perform at a local city theatre in a professional production. The student participants in Study 2 were auditioning for roles in plays that would be produced by fellow students and performed at a university campus theatre. The greater prestige associated with a successful audition in Study 1 may have increased the importance attached to being socially evaluated, and may explain some of the difference in the variable capacity of this context to provoke the HPA axis.

Study 2 comprised a predominantly female sample (n = 12). Sex has been shown to be a key modulating influence on cortisol response to psychosocial stress. In young adults, males have been shown to demonstrate significantly higher salivary cortisol responses to acute laboratory stress compared to females (Kirschbaum, Bartussek et al., 1992; Kumsta, Entringer, Hellhammer, & Wust, 2007; Lovallo et al., 2006; Nicolson, Storms, Ponds, & Sulon, 1997). Variation in salivary cortisol response to acute stress between the sexes is likely related to the divergent internal endocrine milieu. The modulating influence of the menstrual cycle and hormonal contraception use has been emphasised (Kirschbaum et al., 1999; Kudielka & Kirschbaum, 2005). For example, salivary cortisol responses to the TSST have been shown to be modulated by menstrual cycle phase. Women in the luteal phase show comparable salivary cortisol responses to males. Significantly lower salivary responses are demonstrated by women in the follicular phase or taking ethinyl estradiol containing OC's (Kirschbaum et al., 1999; Rohleder, Schommer, Hellhammer, Engel, & Kirschbaum, 2001).

The majority of female participants in this study (n = 10/12) were taking OC's and the remaining participants were in the follicular phase of the menstrual cycle. This may explain the lack of a significant cortisol response in the sample. Oral contraceptive use alters CBG levels. A significant negative correlation between CBG and salivary cortisol response to acute psychosocial stress in women taking OC's has been demonstrated (Kumsta et al., 2007). However, sex cannot be the only factor responsible for the lack of effect on cortisol response since even less of a response was elicited from male participants. Furthermore, all female participants in Study 1 were taking OC's and a significant response was provoked in these participants.

An additional factor that may explain the variability in cortisol response across the two audition contexts is social support. Social support given prior to exposure to the TSST has been shown to attenuate cortisol response in men (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003) and women (Ditzen et al., 2007). Although divergent effects of social support provided by romantic partners have been demonstrated (male attenuated; females augmented; Kirschbaum, Klauer, Filipp, & Hellhammer, 1995). Auditioning participants in Study 1 were members of amateur dramatic societies. Whilst the auditionees were familiar with each other from attending numerous auditions, they did not interact socially outside of audition meetings. Conversely, the performing arts students were class mates and appeared more intimately and socially linked and thus perhaps more likely to provide social support to each other in this situation.

Finally, a number of studies have reported the availability of dietary energy supply exerting regulatory action on HPA axis responses to stress (Gonzalez-Bono, Rohleder, Hellhammer, Salvador, & Kirschbaum, 2002; Kirschbaum et al., 1997). Participants were asked to fast one hour prior to saliva sample collection. However, due to the nature of the naturalistic environment it was not possible to control nutritional status prior to the study.

The failure of the audition context piloted in Study 2 to significantly provoke the HPA axis resulted in the decision not to conduct a follow up repeat study with this stress context. Whilst it would be interesting to further investigate the factors underlying the divergent capacity of the audition context to elicit cortisol responses, the identification of a reliable stress context capable of inducing significant cortisol excursions over repeated exposures was considered a greater priority.

5.5.2 Cardiovascular Response

The audition context elicited a significant cardiovascular response. Significant elevations in SBP compared to non-audition day baseline levels were revealed. However, SBP was significantly elevated pre-audition exposure resulting in less variability in response across the audition day response profile compared to Study 1. In Study 1, the SBP response to stress visit one peaked after audition exposure. This peak was significantly higher than pre-audition measures. In contrast, responses during Study 2 were elevated – 20 minutes prior to audition exposure suggesting a heightened anticipatory response. Significant recovery in SBP by + 20 minutes post-

audition exposure offers further support that the audition context had affected cardiovascular stress parameters. However, the profile suggests a cardiovascular response may have been triggered in advance of – 20 minutes pre-audition onset. Despite significant elevations above non-audition day baseline levels, DBP did not show significantly changes across the profile on the audition day. Peak response was – 20 minutes prior to audition onset which again suggests the response may have been initiated prior to measures being taken. Greater cardiovascular responsivity in males was again demonstrated supporting the findings of Study 1 and examples in the research literature (Matthews et al., 2001; Steptoe et al., 1996).

5.5.3 Subjective Stress Response

The audition context was sufficient to induce changes in subjective stress and mood responses. Subjective stress ratings were significantly higher than the non-audition day ratings. During exposure to the audition subjective stress peaked immediately prior to audition onset and only declined to comparable non-audition day levels 30 minutes post-audition onset. The subjective stress response profile was comparable to that demonstrated during Study 1 (Study 1 peak, $X = 50.30 \pm 3.00$; Study 2 peak, $X = 48.44 \pm 2.90$). However, the peak in response during the current study occurred prior to the audition with a pronounced decline once the audition was over. During Study 1 a comparable subjective stress peak was demonstrated immediately pre- and post-audition exposure. A comparable mood response profile to that shown in Study 1 was again demonstrated for Subjective POMS ratings of Tension-anxiety and Vigour-activity. Additionally, participants reported significant Fatigue-inertia after the audition.

A lack of cortisol response in the presence of heightened subjective indicators of stress emphasises desynchrony between subjective and endocrine stress responses. Minimal correlation between cardiovascular and subjective perception of stress supports this point. Study 1 revealed stronger correlation between the level of social-evaluative threat and cortisol response compared to the relationship between subjective perception of stress and cortisol. This finding was not replicated here.

5.5.4 Cognitive Performance

Audition exposure impaired WM performance. Two-back target and overall accuracy and A-ospan total were impaired. This finding replicates studies which reported impaired WM performance under stress conditions (Elzinga & Roelofs, 2005; Luethi et

al., 2009; Schoofs et al., 2008; Schoofs et al., 2009). In contrast to these studies, the present study reports impaired performance in the absence of a significant cortisol response. The key role of cortisol has been previously emphasised with cortisol levels accounting for approximately 20% of the variance in WM performance (Oei et al., 2006; Schoofs et al., 2009). Despite evidence that moderation of cognitive function may require concurrent cortisol and adrenergic arousal (Elzinga & Roelofs, 2005; Tollenaar et al., 2008), cortisol is often reported to be the primary variable in the relationship. Therefore this finding may be considered surprising. The effects of stress on cognitive performance may operate in an inverted-U function. Suboptimal levels of cortisol may have underpinned impaired cognitive performance in this sample. Animal models have demonstrated that cortisol-modulated effects on cognitive performance may be largely determined by the ratio of MR and GR GC receptor saturation (de Kloet et al., 1999). The MR/GR receptor ratio hypothesis suggests memory performance can be enhanced under conditions in which the majority of MR and only a small proportion of GR GC receptors are occupied. Conversely, a significant decrease or increase of GC receptor occupation may result in impaired cognitive performance. Lupien et al. (1999) have previously demonstrated a U-shaped curve relationship between WM performance and GC levels following hydrocortisone infusion. Psychosocial stress studies that failed to elicit significant elevations in cortisol response have reported no effect (Hoffman & Al'Absi, 2004; Smeets et al., 2006) or enhanced effects (Duncko et al., 2009) of stress on WM performance. However, it is difficult to disentangle potential effects of cortisol levels from divergent methodological variables (e.g., tests of WM, time of day, and stress induction method).

Impairment of WM function in the absence of GCs may be underpinned by noradrenergic arousal modulating PFC-dependent cognitive functions (Robbins, 2005; Usher, Cohen, Servan-Schreiber, Rajkowski, & Aston-Jones, 1999). Rapid activation of the SNS following exposure to acute stress results in the release of NE from a widely distributed neural network of synapses including those that project to the PFC (Robbins, 2000). Animal models have demonstrated that catecholamines acting on the PFC exert an inverted U-shaped influence on cognitive performance with sub- or supraoptimal levels impairing WM function (Arnsten, 1998; Birnbaum, Podell, & Arnsten, 2000). On a longer timescale, acute stress can activate the release of GCs which may act to exacerbate the impairing effect of supraoptimal catecholamine levels on cognitive function (Qin et al., 2009). The impaired WM function reported here may have been underpinned by catecholamines acting upon the PFC. However, in the

absence of a direct measure of catecholamine activation, this interpretation cannot be confirmed.

Research examining the effect of acute stress on cognitive function has predominantly focussed upon physiological mechanisms of impairment. A number of psychological mechanisms have been proposed to account for impaired cognitive performance under stress that emphasise the importance of resource competition (Sliwinski, Smyth, Hofer, & Stawski, 2006). Stress is proposed to impair cognitive function by limiting the cognitive resources available for information processing. In this sense, stress operates as a cognitive load resulting in limited attention being divided between the demands of the cognitive task and coping with the psychological demands of the stressful context. For example, cognitive interference, underpinned by intrusive thoughts and suppression of intrusive thoughts related to the stress context, has been shown to impair WM (Klein & Boals, 2001b), and episodic memory and processing speed (Stawski, Sliwinski, & Smyth, 2006, 2009). Klein and Boals (2001a) have also demonstrated that a stress-reduction intervention designed to reduce cognitive interference improved WM function. Future studies in this thesis therefore include a measure of cognitive interference to examine the potential for psychological mechanisms to modulate cognitive performance. These measures were included to provide further insight into the mechanisms underpinning cognitive performance under stress; particularly in the absence of a cortisol response as reported here. Impaired cognitive performance associated with psychological factors remains relevant to a PL intervention considering previous evidence of the capacity of PL supplementation to attenuate psychological stress responses (Benton et al., 2001; Hellhammer et al., 2004).

5.5.5 Potential Predictors of Cortisol Responsivity

Exploratory analysis of potential predictors of cortisol response undertaken on the combined sample from Study 1 and 2 provided further support for perfectionism as a promising trait in the identification of a sample of stress vulnerable participants. Despite limited cortisol responsivity in the majority of Study 2 participants included in the regression models, Perfectionism: Organisation showed similar predictive power to that demonstrated in Study 1, and explained significant proportions of the variance in additional measures of cortisol responsivity. Perfectionism: Organisation remained a significant predictor of salivary cortisol AUCg and levels at + 10 and + 20 minutes relative to audition onset. This perfectionism dimensional trait also explained a

significant amount of the adjusted variance in cortisol AUCi, delta increase, and levels at + 30 minutes.

Perfectionism: Organisation emerged as the only significant predictor of adjusted variance in cortisol delta increase. Delta cortisol increase can be considered an index of responsivity (peak amplitude). The relationship between Perfectionism: Organisation and this index of cortisol response may indicate that perfectionism is related to heightened cortisol responsivity. However, as this relationship only emerged after the addition of the Study 2 sample, within which only a minority of participants demonstrated variability in cortisol responsivity, this effect may be a function of the small number of participants. Continued examination of the relationship between trait variables and cortisol response in subsequent studies presented in this thesis that provoke significant cortisol responses was undertaken to further elucidate this relationship. Age and sex emerged as significant predictors of cortisol levels at + 30 minutes which may suggest a role in cortisol recovery. Whilst the combined sample size was still modest (N = 33), perfectionism remained the only trait variable that demonstrated any predictive relationship to measures of cortisol response.

5.6 Interim Summary

Study 2 demonstrated the inconsistency inherent in testing stress responses in naturalistic environments. The audition context was sufficient to impact upon cardiovascular and subjective parameters of stress but failed to provoke the HPA axis. Cortisol responsivity is highly variable in both activation and sensitivity to time-constant and time-varying mediating and moderating factors. Hence, the lack of control and standardisation inherent in naturalistic studies increases the likelihood of such factors influencing study outcomes. The identification of candidate stress-sensitive cognitive tests (albeit not as a direct function of cortisol), and further support for trait factors predictive of cortisol responsivity, contributed to fulfilling the methodological aims for the dietary intervention study (Chapter 7). These measures were taken forward into Study 3

Chapter 6:
Study 3

Chapter 6 Study 3 - Stress Responses and Cognitive Performance Following Repeated Exposure to a Laboratory Stressor

6.1 Introduction

The psychological attributes of specific stressful contexts appears to be a key factor in the provocation of the HPA axis (Dickerson & Kemeny, 2004; Mason, 1968; Rose, 1984). The audition exposure in Study 2 was a subjectively stressful experience that was comparable in terms of timing, form, and structure to the audition exposure examined in Study 1. However, an undefined variable or variables resulted in this context not being sufficient to elicit a cortisol response in Study 2. Research has elucidated numerous moderating and intervening factors that significantly influence the response of the HPA axis to stress. Indeed, considerable inter- and intra-individual variance across stress contexts is a prominent characteristic of the salivary cortisol response to acute stress provocation. The use of naturalistic stress contexts or ambulatory assessments offers the advantage of examining stress responses, and cognitive performance, under conditions of genuine psychophysiological stress that are likely to be more intimately linked to the goals, interests, and identity of the individual. However, the increased ecological validity of a naturalistic stress setting inevitably comes at the cost of the capacity to control potential confounding variables.

Considering the numerous potential variables that have been shown to influence salivary cortisol response to acute stress exposure (see Kudielka et al., 2009 for a review; discussed in Sections 1.3.1; 1.3.2), the increased control and standardisation offered by laboratory stress protocols may be likely to produce more consistent stress responses than those observed in Studies 1 and 2. Laboratory protocols offer the benefit of greater control over variables that may affect individual responses to acute stress. For example, the level of social-evaluative threat can be standardised across participants and exposures. Studies 1 and 2 demonstrated the inconsistency in the capacity of a naturalistic social-evaluative stressor to provoke a cortisol response. In contrast, the laboratory-based TSST has consistently demonstrated cortisol responder rates of over 70% (Kudielka, Hellhammer, & Kirschbaum, 2007). Hence, a laboratory stress protocol was piloted in Study 3.

Evidence of the slow accumulation of PL in the brain (Bruni et al., 1989; Pepeu et al., 1996) suggests chronic administration of PL may be required for any observed effects.

Furthermore, cortisol response habituation to repeated exposure to laboratory stress separated by four weeks or less has been reported (Gerra et al., 2001; Kirschbaum, Pruessner et al., 1995; Schommer et al., 2003; Wust, Federenko, Van Rossum, Koper, & Hellhammer, 2005a). There is modest evidence to suggest longer delays between stress exposures may be sufficient to reduce the level of habituation in cortisol response. Kirschbaum has previously stated that unpublished data from his laboratory demonstrates that a delay of four months between TSST exposures and contextual changes is sufficient to prevent significant habituation (Kirschbaum and Foley, 2010). Furthermore, Petrowski et al. (2012) reported that an interval of 10 weeks was sufficient to reinstate previously habituated cortisol responses. This would suggest a relatively chronic intake of PLs and a delay between repeated stress exposures in excess of four weeks would be advantageous. An inter-stressor delay of six weeks was therefore adopted in Study 3 in acknowledgement of such evidence.

6.1.1 Control of Sex Differences

There is ample evidence to suggest that males and females differ in how they respond behaviourally and endocrinologically to acute stress (Kirschbaum et al., 1999; Kudielka, Buske-Kirschbaum et al., 2004; Kudielka, Hellhammer, & Wust, 2009; Kudielka & Kirschbaum, 2005; Levine, Zagoory-Sharon, Feldman, Lewis, & Weller, 2007; Taylor et al., 2000). Evidence of the modulatory impact of the menstrual cycle and OC use suggests the gold standard study design for examining HPA axis-mediated responses in mixed samples would be to test women in the luteal phase (Kudielka, Hellhammer, & Wust, 2009). Indeed, comparable salivary cortisol responses have been reported between males and females in this cycle phase (Kirschbaum et al., 1999; Rohleder et al., 2001). However, the prevalence of OC use in young women creates difficulties in recruiting such a sample; especially if age-matching is required. Greater male cortisol responsivity and the subsequent predominantly male impairment of WM performance have also been reported even when this control measure has been employed (e.g., Elzinga & Roelofs, 2005). Furthermore, in contrast to men, women in the luteal phase demonstrate reduced glucocorticoid sensitivity which may lessen the impairing effects of stress on memory (Rohleder et al., 2001; Schoofs & Wolf, 2009). Evidence also suggests that men and women differ, not only in endocrine responses, but also in the effect of stress or synthetic glucocorticoid administration on memory processes (Wolf, 2003). Studies examining the effects of sex on cognitive performance under stress have shown

greater declarative memory impairment (Wolf et al., 2001) and fear conditioning (Zorawski et al., 2005) in males compared to females.

Whilst the need to examine effects of a nutrient intervention in a mixed sex sample to fully assess the stress-buffering potential effects of PLs is acknowledged, the sample size required to fully explore and/or control for the modulating effects of menstrual cycle phase/OC use was beyond the scope of the intervention study. Furthermore, the PL intervention period was planned to be in excess of four weeks to increase the potential for the active ingredients to exert a functional effect. This would, therefore, create difficulties matching female participants for menstrual cycle phase pre- and post-intervention, compounded by variability in cycle length and regularity between and within female participants (Chiazze, Franklin, Maciso, Parker, et al., 1968; Sherwood, 2004). Accordingly, a male only sample was recruited for Study 3 with the intention of undertaking the subsequent PL intervention study (Study 4) on this subset of the population.

6.1.2 Identification of a Laboratory-Based Stressor

Whilst increased levels of control and standardisation in laboratory psychosocial stress protocols increase consistency in response provocation, significant habituation in cortisol responses across repeated exposures is often demonstrated in the majority of individuals (Gerra et al., 2001; Kirschbaum, Pruessner et al., 1995; Schommer et al., 2003; Wust, Federenko, Van Rossum, Koper, & Hellhammer, 2005b). Comparing stress responses and cognitive performance before and after a nutritional intervention in the absence of comparable cortisol responsivity across repeated exposures increases the likelihood that any effect of the nutritional manipulation will be confounded, or overshadowed, by divergence in the background endocrine milieu. Consequently, previous evidence of specific elements of laboratory stressors associated with cortisol responsivity and response habituation was used to inform the design of the laboratory-based repeated stressor study reported in this chapter.

The social-evaluative threat aspect of the TSST appears crucial to the capacity of this stress protocol to reliably provoke significant elevations in salivary cortisol (Dickerson & Kemeny, 2004). Hence, this protocol was adopted as the basis of the stress protocol administered in Study 3. Speech tasks have been previously demonstrated to elicit larger and more consistent endocrine (ACTH and cortisol) and cardiovascular responses than mental arithmetic tasks (AlAbsi et al., 1997). Hence, the TSST speech

task was retained. However, in an attempt to reduce the habituation commonly demonstrated in response to this stress protocol, a number of changes were made.

In addition to the classic socially evaluated speech aspect of the TSST, a physical stressor in the form of a socially evaluated cold pressor test (SECPT; Schwabe, Haddad, & Schachinger, 2008) was added to the stress procedure (described in detail in Section 6.3.10.1). Animal models suggest less rapid HPA axis response habituation to physical, rather than psychological stressors; particularly those involving a proximate physical threat (see Grissom & Bhatnagar, 2009 for a review). A similar combination of socially-evaluated psychosocial and physical stressors has been administered without significant habituation in salivary cortisol response in humans (Sheila West, 2012, personal communication). Furthermore, physical stressors such as the SECPT, are potent stimulators of the SNS (al'Absi, Petersen et al., 2002). Considering the evidence of the key role of synergistic HPA axis and SNS arousal in the impairment of cognitive function, the combination of a potent agitator of the HPA axis (TSST speech), and the SNS (SECPT), were considered crucial to developing a reproducible stress response in line with the aims of this thesis.

Novelty, lack of control, unpredictability, and social-evaluative threat have been identified as primary psychological determinants of cortisol responsivity to acute psychosocial stress (Dickerson & Kemeny, 2004; Mason, 1968; Rose, 1984). Repeated exposure to a homotypic stress protocol seems likely to reduce stressor novelty, and increase perceived controllability and predictability. Increased familiarity, control and predictability may also reduce the impact of any social evaluation experienced during stress exposure. Familiarity with a stress context and increased behavioural control over the experimental situation is associated with reduced responsivity (Harl et al., 2006; Voigt et al., 1990). For example, decreased HPA axis reactivity over repeated stress exposure has shown to be accompanied by increasing perceived controllability and decreasing perception of novelty and unpredictability (Schommer et al., 2003). Contextual changes to a homotypic stressor may ensure that the perceived novelty, uncontrollability, and unpredictability of the initial exposure are not significantly diminished by the second exposure. Consequently, contextual aspects of the stress protocol (speech task, stress room, experimenter, and social-evaluative panel) were changed across stress exposures (described in detail in Section 6.3.10.1).

As an additional manipulation to increase the potency of the stress protocol, female social-evaluative panel members were employed. Increasing evidence suggests that opposite sex effects are important in elicitation of endocrine, sympathetic, and subjective stress responses to psychosocial stress (Larkin, Ciano-Federoff, & Hammel, 1998; Martinso & Zerface, 1970; Roney, Lukaszewski, & Simmons, 2007; Roney & Simmons, 2008; Roney, Simmons, & Lukaszewski, 2010). Duchesne, Tessera, Dedovic, Engert, & Pruessner (2012) reported that males, and females in the follicular phase, demonstrate significantly greater cortisol, BP and subjective stress responses to opposite vs. same sex TSST social-evaluative panels. Furthermore, males demonstrate increased cortisol activation after contact with women they find attractive (Van der Meij, Buunk, & Salvador, 2010).

6.1.3 Predictors of Cortisol Responsivity

A sub-dimension of perfectionism emerged as the only significant predictor of cortisol responsivity to psychosocial stress in Studies 1 and 2. However, evidence suggests that trait personality factors related to response disposition may only emerge over aggregated stress exposures (Kirschbaum, Bartussek et al., 1992; Pruessner, Gaab et al., 1997). Epstein (1986) proposes the examination of repeated stress exposures may reveal a cross-situational stable component underlying cortisol responsivity that may be masked by the initial novelty associated with a stressor. Furthermore, a high-responder sub-set of the population has been shown to demonstrate heightened cortisol responses over repeated psychosocial stress exposures (Gerra et al., 2001; Kirschbaum et al., 1995). Measurement of responses and trait characteristics over repeated stress exposures in Study 3 permitted further examination of potential predictors of cortisol response to laboratory psychosocial stress, and to establish whether any hitherto non-significant trait predictors emerged over aggregated repeated stress exposures.

6.1.4 Nutritional State

The increased cortisol secretion profile typically demonstrated after psychosocial laboratory stress exposure has been shown to be modulated by nutritional status and macronutrient intake. Kirschbaum et al. (1997) showed that participants given a 100g/400ml glucose load one hour prior to stress exposure demonstrated the typical increased cortisol response. However, participants who were fasted prior to stress exposure failed to show this response. Subsequent research has demonstrated

greater absolute and net cortisol increase following glucose intake compared to fat or protein administration (Gonzalez-Bono et al., 2002). A positive correlation between blood glucose and cortisol was also reported. Furthermore, intranasal administration of insulin has been shown to attenuate cortisol response to the TSST (Bohringer, Schwabe, Richter, & Schachinger, 2008).

It is assumed that a central, rather than peripheral, mechanism underlies the regulation of energy balance and HPA axis activation (Kudielka, Hellhammer, & Wust, 2009). However, the inability to instigate a cortisol response at low euglycemic ranges is at odds with the traditional view that the primary function of HPA-axis activation under stress is the liberation of energy substrates via gluconeogenesis. In a review of the literature, Rohleder & Kirschbaum (2007) concluded that available data/studies are not sufficient to explain the relationship between nutritional state and acute cortisol responses. Whilst the mechanism is yet to be fully elucidated, such evidence suggests that standardisation of nutritional state prior to studies, of which cortisol is a primary dependent variable, would be a beneficial addition to study designs. The increased level of control of a laboratory study permitted the standardisation of nutritional state by administration of a standardised study meal in Study 3 (described in Section 0).

6.1.5 Identification of Cognitive Tests

The 2-back and A-ospan cognitive tests were shown to be sensitive to the impairing effects of stress in Study 2. Therefore, these tests were carried forward into Study 3 as candidate tests for the PL intervention study. It should be noted that due to the limitations of the Study 2 naturalistic testing environment, it was not possible to conduct a non-audition control day. Participants completed three trials of each WM test on the non-audition day with the third trial used in subsequent stress vs. non-stress analysis. Three consecutive completions of the tests on the non-audition day may have inflated performance levels compared to the post-audition performance during which participants completed the test only once. Whilst evidence suggests performance reaches an asymptote by the third repeat trial (Bartels et al., 2010), it was thought that comparing performance across a matched non-stress control day comparator would shed light on the sensitivity of these tests to the effects of stress. The greater level of control possible in a laboratory-based study allows for the completion of a non-stress control day to compare cognitive performance and stress responses across conditions differing only in the induction of stress.

Two additional cognitive tests were selected for piloting in Study 3. The VISGED (Etzel & Hornke, 1999) is a test of visual memory that has been previously shown to be sensitive to a PL manipulation in a subset of older high stress-load men exposed to a TSST (Schubert et al., 2010). A task-switch test was also piloted. The ability to switch between tasks is considered a measure of executive function/control. There is emerging evidence in animals (Butts et al., 2013) and humans (Konishi et al., 1999; Plessow et al. 2011) that this aspect of PFC-mediated cognitive performance is vulnerable to stress.

Evidence of impaired cognitive performance in the absence of a significant cortisol response highlights the potential contribution of other variables to the stress-cognition relationship (previously discussed in Section 5.5.4). Consequently, measures of cognitive distraction/interference were included in Study 3 to ascertain whether cognitive factors might also influence performance (see Section 6.3.6.5).

6.2 Study Objectives and Hypotheses

Study 3 explored the feasibility of utilising a laboratory-based stressor characterised by social-evaluative threat for the planned PL intervention study (Study 4). Exposure to the laboratory stressor was expected to induce significant elevations in salivary cortisol, cardiovascular, and subjective stress reactivity above baseline levels in males. The manipulation of contextual and psychosocial variables across Stress Visits 1 and 2 and the addition of a physical stressor were expected to reduce habituation in cortisol response to repeated stress exposure. Working memory performance (2-back and A-ospan) was expected to be impaired, relative to the non-stress control, following exposure to the laboratory stressor repeating evidence of the sensitivity to impairment by stress demonstrated in Study 2. Performance on the VISGED and task-switch task was also expected to show impairment under conditions of stress exposure relative to non-stress control.

6.3 Methods

6.3.1 Sample

Twenty-five healthy, young male participants were recruited via the University of Leeds participant database and recruitment posters displayed on campus. Eligibility was determined during an initial screening visit at the Institute of Psychological

Sciences (details given in Section 6.3.2). Participant characteristics are reported in Section 6.4.1.

6.3.2 Inclusion and Exclusion Criteria

A screening visit was undertaken to ensure the following study inclusion/exclusion criteria were met:

6.3.2.1 Inclusion criteria

- Male over 18 years of age

6.3.2.2 Exclusion criteria

In addition to those stated in Methodologies Section 3.3.4:

- Medication use (prescribed and 'over-the-counter'). Skin conditions (e.g. eczema) on hands (due to SECPT)
- Food allergies
- Participation in a clinical study within a month prior to screening or during participation
- Previous participation in a stress induction study

6.3.3 Design

The study conformed to a repeated measures design comprising both a non-stress control visit and a stress visit in week one (separated by no more than 3 days), and a repeat stress visit after a six week delay. Stress Visit 1 and the non-stress control day (week one) were counterbalanced to account for potential order effects influencing performance on cognitive tasks. Stress Visit 2 was completed six weeks after completion of Stress Visit 1. Cognitive performance and acute physiological/subjective stress responses under control, stress, and delayed repeated stress conditions were examined. The study flow from screening to completion is shown in Figure 6.1.

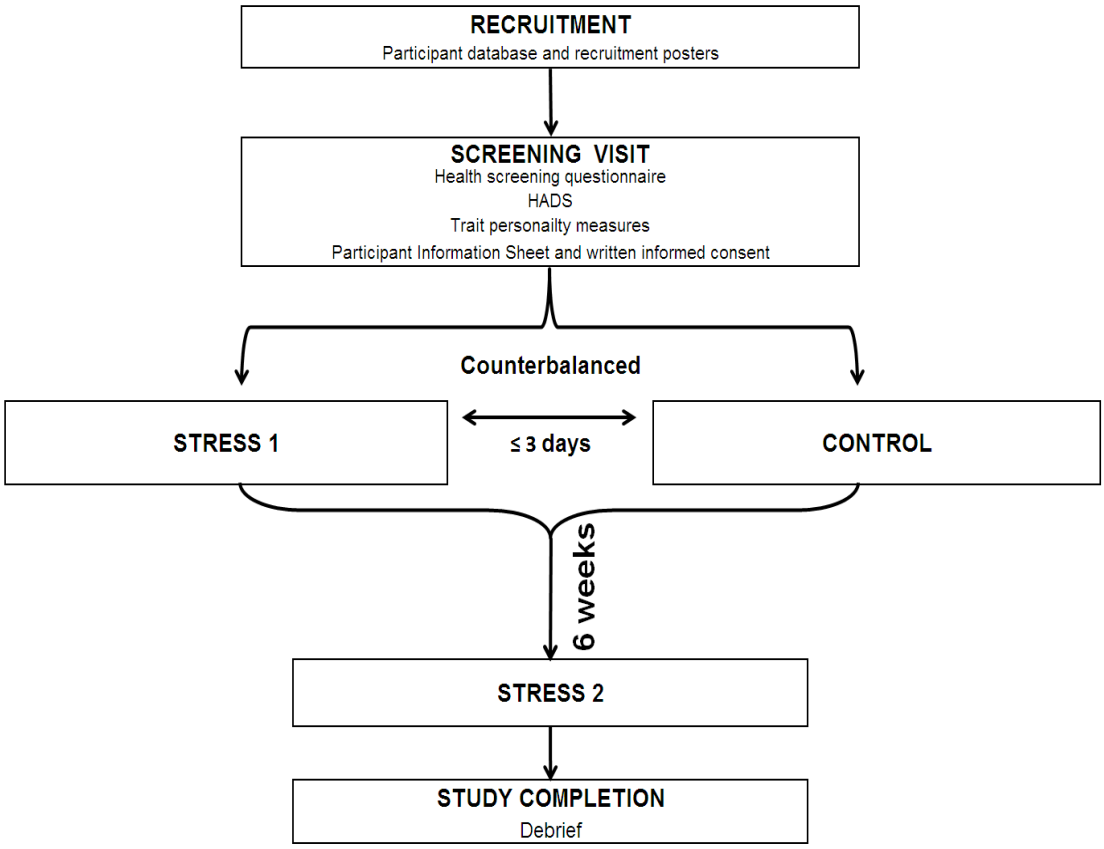


Figure 6.1 Study flow diagram

6.3.4 Endocrine Measures

6.3.4.1 Cortisol

Salivary cortisol samples were collected using a Salivette® device (Sarstedt, Numbrecht, Germany) at 0, + 10, + 25 (mid-stress), + 35, + 50, and + 60 minutes relative to visit commencement across all study visits. Salivary-free cortisol concentrations were determined using a Salivary Cortisol Enzyme Immunoassay kit (EIA; Sarstedt; Nümbrecht, Germany; described in Section 3.2.1.1). Intra- and inter-assay variability was below 4.5 and 10.36% respectively.

6.3.5 Physiological Measures

6.3.5.1 Blood pressure

A Spacelabs (model 90207) ambulatory blood pressure monitor was used to measure SBP, DBP, and HR at the brachial artery of the upper (non-dominant) arm at 0, + 10, +

20 (pre-speech), + 25 (mid-stress), + 30, + 35, + 50, and + 60 minutes relative to visit commencement across all study visits. To account for potential variability in blood pressure monitor reading two measurements were taken at each time point and the average of the readings employed in all analyses.

6.3.5.2 Capillary blood glucose

Glucose levels in capillary whole blood samples were measured using a Glucomen® LX meter (Menarini Diagnostics, UK). Glucomen ® LX meters require 0.3 µL of capillary blood taken from the finger extracted using a lancet finger prick device. The measuring range of the device is 1.1 – 33.3 mmol/L. Glucose was sampled + 10 minutes and + 35 minutes relative to visit commencement across all study visits.

6.3.6 Subjective Measures

The following measures were employed to assess baseline chronic stress level and acute subjective responses to stress and control exposures. More detailed descriptions of these measures are given in the General Methodologies Section (Chapter 3) where indicated.

6.3.6.1 Perceived Stress Scale (PSS)

Participants completed the first PSS at the face to face screening ≤ 5 days prior to Stress Visit 1. A second PSS was completed at Stress Visit 2 to explore for potential differences in background stress levels across the repeated stress visits (General Methodologies Section 3.3.1)

6.3.6.2 Stress and Arousal Checklist (SACL)

The SACL was completed at 0, + 10, + 35, +50, and + 60 minutes relative to visit commencement across all study visit (General Methodologies Section 3.2.3.1).

6.3.6.3 Profile of Mood States Short Form (POMS-SF)

The POMS-SF was completed at 0, + 10, + 35, +50, and + 60 minutes relative to visit commencement across all study visits (General Methodologies Section 3.2.3.2).

6.3.6.4 Primary Appraisal Secondary Appraisal (PASA)

The PASA was completed at + 10 minutes relative to visit commencement across all study visits (General Methodologies Section 3.2.3.4)

6.3.6.5 Cognitive distraction/interference VAS

Four cognitive distraction/interference VAS were employed to explore the potential influence of cognitive factors on cognitive performance (Appendix 20). The VAS were adapted from the Likert scales employed by Stawski et al. (2009). Two VAS, administered across the control and both stress visits, assessed general levels of distraction ("How distracted by your thoughts did you feel while you performed the previous memory tasks?") and intentional suppression of off-task thoughts (How much did you feel yourself intentionally suppressing off-task thoughts while you were performing the memory tests?") experienced whilst completing the cognitive tests. Two further VAS assessed cognitive distraction and interference related to the experimental context. During the control visit the VAS assessed cognitive interference/suppression of off-task thoughts related to the general testing scenario (e.g., "How distracted did you feel by thoughts about the test scenario while performing the memory tasks?"). During the stress visits the VAS assessed cognitive interference/suppression of off-task thoughts related to stress exposure (e.g., "How distracted did you feel by thoughts about the challenging tasks while performing the memory tests?"). Responses were made using 100 mm VAS anchored by the descriptors "*Not at all*" and "*A lot*". Cognitive interference VAS were completed at + 70 minutes after completion of cognitive tests.

6.3.7 Trait Personality Measures

The following trait personality measures were employed in order to explore potential trait predictors of acute cortisol responsivity to stress. More detailed descriptions of all personality measures are given in the General Methodologies Chapter 3 (sections shown in parentheses). The reliability coefficients for each measure are given here. All personality measures were completed at screening.

- Frost Multidimensional Perfectionism Scale (Section 3.4.1). Acceptable reliability coefficients were found for all subscales: *Parental Expectation* (r_α = .72), *Organisation* (r_α = .91), *Parental Criticism* (r_α = .68), *Concern over*

Mistakes (r_α = .89), *Personal Standards* (r_α = .88), *Doubts about Actions* (r_α = .72), and *Total Perfectionism Score* (r_α = .91).

- Rotter's external-internal Locus of Control Scale (Section 3.4.2; r_α = .80)
- Rosenberg Self-Esteem Scale (Section 3.4.3; r_α = .74)
- Neuroticism (IPIP-N; Section 3.4.4; r_α = .69)
- Toronto Alexithymia Scale 20 Item Version (Section 3.4.5). Acceptable reliability coefficients were found for the alexithymic subscales of *Difficulty Describing Feelings* (r_α = .80) and *Difficulty Identifying Feelings* (r_α = .78). The subscale *Externally-Orientated Thinking* was again found to be less reliable (r_α = .63). Comparable inconsistent reliability coefficients have been previously reported for this alexithymic subscale in Study 2 (r_α = .66; Section 5.3.8) and the literature (Bagby et al., 1994; Bressi et al., 1996; Loas et al., 2001).
- State-Trait Anxiety Inventory (Section 3.4.6; r_α = .82)
- Cook-Medley Hostility Scale (Scale; Section 3.4.7; r_α = .79)

6.3.8 Standardised Test Meal

A fixed meal was given to participants prior to each test session to standardise nutritional status. A tomato and vegetable risotto rice ready meal (Uncle Bens®) was served to participants upon arrival (see Table 6.1). The one hour relaxation period prior to collection of the first study measures (0 minutes) commenced immediately after the consumption of the test meal. The test meal was well tolerated and fully consumed by all participants on each test day.

Table 6.1 Nutritional values per 125g serving of the standardised test meal

	Per 125g serving
Energy	944 KJ
Kcal	224kcal
Carbohydrates	39.1g
of which sugars	2.4g
Protein	4.6g
Fat	5.1g
Sodium	0.41g
Fibre	1.6g

6.3.9 Tests of Cognitive Performance

A full description of each cognitive test is detailed in the General Methodologies Chapter 3 (sections shown in parentheses).

6.3.9.1 2-back

Three trial blocks of 50 stimuli were presented to participants. Digits were presented for 500 ms with an inter-stimulus delay of 1000 ms (Section 3.5.3.1).

6.3.9.2 A-ospan

Six stimulus blocks of 3 and seven stimulus blocks of 4 set size operations (13 operations in total). The maximum score possible for A-ospan score and total was 46 (Section 3.5.3.2).

6.3.9.3 VISGED

The screening version of the VISGED (Section 3.5.3.3) was completed as a measure of visual memory.

6.3.9.4 Task-switch test

A task-switch test was employed as a measure of executive control (Section 3.5.3.4).

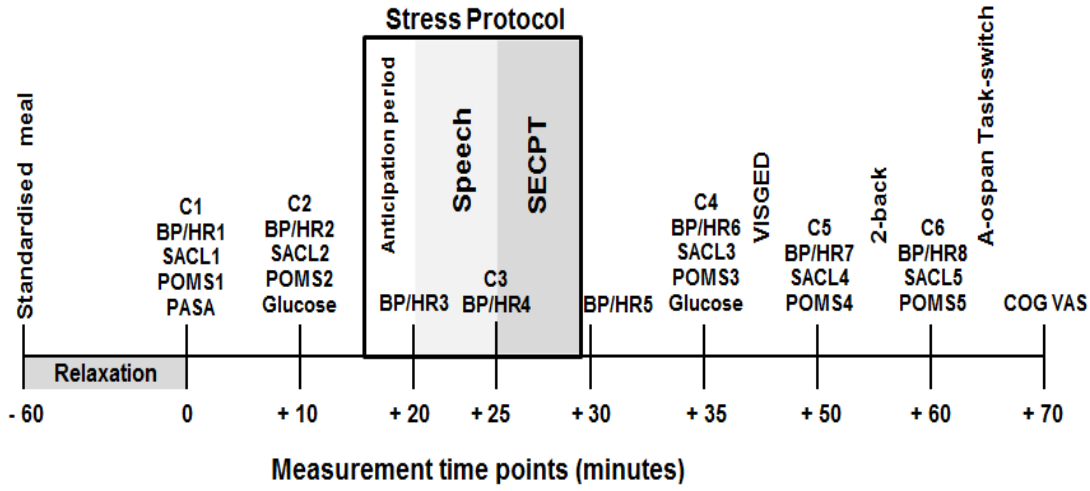
6.3.10 Procedure

Participants attended an initial screening visit during which they were familiarised with the study measures. Participants read the study participant information sheet and provided written informed consent. Four BP/HR measures were collected to exclude participants exhibiting raised BP (> 140/90 mmHg over repeated measures). The 2-back, A-ospan and task-switch test were completed twice during the screening visit. The VISGED was completed once. Trait personality measures were also completed and body weight and height was measured (to calculate BMI).

The control and both stress visits were matched in terms of the timing and collection of all study measures. All study visits commenced between 1100 hr and 1600 hr (all cortisol measures and cognitive testing therefore occurred between 1200 hr and 1720 hr). A standardised test meal and water were consumed upon arrival at the Institute of Psychological Sciences. Participants then relaxed in a testing cubicle for one hour

after consumption of the meal. After completion of the relaxation period an ambulatory BP monitor was fitted to the upper non-dominant arm of each participant. Salivary cortisol, cardiovascular and subjective response measures (SACL and POMS) were collected at timed intervals across each visit (see stress visit procedural timeline for specific measurement time points; Figure 6.2). Capillary blood glucose measures were collected + 10 and + 35 minutes relative to visit commencement across all study visits. Participants were introduced to the first stress task at + 15 minutes. The stress induction protocol is outlined in Section 6.3.10.1. Briefly, the stress induction procedure comprised introduction to the speech task followed by a 5 minute anticipation period. The SECPT task was completed following the delivery of the speech. Both stress tasks were completed in a stress induction room in front of an evaluative panel. Contextual changes were made across Stress Visits 1 and 2 (detailed in Section 6.3.10.1) to reduce the potential for habituation. Participants relaxed in the testing cubicle during this period on non-stress control visits. To match the physical exertion of walking to the stress induction room participants were instructed to walk to this room and back on the control day. Following completion of the stress induction/non-stress control period the cognitive tests were completed in serial order. Cortisol, cardiovascular and subjective measures were collected in between each cognitive test. The cognitive interference/suppression VAS were administered after all cognitive tests were completed (+ 70 minutes).

A partial debrief was given to participants following the completion of Stress Visit 1 explaining that none of the 'recorded' data would be analysed until completion of Stress Visit 2. Participants returned six weeks (± 2 days) later to complete Stress Visit 2. The start time of the control and stress visits was matched within 1 hour within participants to control for time of day effects. A full debrief was provided upon completion of Stress Visit 2.



C - Salivary cortisol, SACL - Stress and Arousal Checklist, POMS - Profile of Mood States, SECPT - Socially evaluated cold pressor test, Glucose - Fingerprick capillary glucose, PASA - Primary Appraisal Secondary Appraisal

Figure 6.2 Stress visit procedural time

6.3.10.1 Stress Induction

The standard operating procedures for the combined speech/SECPT detailing all standardised instructions are shown in Appendix 21. The stress induction protocol combined the speech task of the TSST (Kirschbaum, Pirke et al., 1993) and the SECPT. Participants were taken to a separate stress induction room to undertake the stress protocol. In this room two female confederates were seated behind a desk on which were placed a video recorder, microphone, and audio recorder. Stress panel members were instructed to remain neutral throughout procedure and offer no verbal or non-verbal reassurance. Participants were directed into the room by the primary researcher and asked to stand in front of the panel. A standardised script detailing the speech task was read to the participant by the primary researcher. The first task required participants to give a 5 minute speech presenting themselves as a job applicant for a management trainee position within the university. The participants were told their performance would be video and audio recorded for analysis and the stress panel would be assessing their verbal and non-verbal behaviour. Participants were informed they would undertake a second challenging task after completing the speech. If the participant agreed to continue with the stress induction they were taken back to the study cubicle and told they had 5 minutes to prepare their speech.

During the 5 minute speech anticipation period the primary researcher prepared the ice bucket for the SECPT. An insulated bucket was filled with 550g of ice and 1 L of

cold water. A thermometer was used to ensure each ice bucket was between 0 – 4 º C. The ice bucket was placed on a low table at the back of the stress induction room. A chair was placed next to the table facing the panel and camera (diagram of room set-up shown in Appendix 21).

The primary researcher returned to the study cubicle and measured the participants BP and HR (+ 20 minutes). If the participant had made notes they were informed they were not permitted to take these into the stress induction room. The participant was led into the stress induction room and directed to stand directly in front of the panel and camera. The primary researcher exited the room. A stress panel member would turn on the video and audio equipment and invite the participant to begin their speech. The stress panel closely observed the participant during the speech and took notes. If the participant paused or asked a question of the panel, a series of scripted replies were given after a pause of 20 seconds (e.g., “you still have time, please continue”). Once the panel had timed 5 minutes the participant was told to stop and the primary researcher returned into the room. The participant was told to take a seat next to the ice bucket. The primary researcher collected + 25 minutes BP, HR, and cortisol measures. The SECPT task was then explained. The participant was directed to place their hand above their wrist into the bowl of ice water for as long as they could whilst looking directly into the camera. The panel would observe the participant for non-verbal behaviour and ‘facial expression analysis’. Participants were told to remove their hand if they had kept it in the ice bucket for 3 minutes. The + 35 minutes BP and HR measures were taken immediately after completion of the SECPT. The participant was then directed back to the study cubicle.

A number of contextual changes were made to the stress induction protocol across Stress Visits 1 and 2. The primary researcher, panel members, stress induction room and speech tasks were changed across each visit. For Stress Visit 2 participants were asked to present their character and personality to the panel including at least one negative and one positive aspect about themselves (Appendix 21 for standardised instructions). Participants were not explicitly told what Stress Visit 2 would entail exactly, only that they would complete two challenging tasks again.

6.3.11 Ethical Approval

All participants provided written informed consent prior to study inclusion. An initial outline of the study was provided to potential participants in an email circulated via the

IPS participant database and to those replying to study recruitment posters. Eligible individuals were invited to a screening visit at the Institute of Psychological Sciences to re-read the participant information sheet and ask any questions about participation. The exact nature of the stress protocol was withheld from participants until the task was to be undertaken. This was deemed necessary, as knowledge of the stress procedure prior to exposure is likely to impact upon the engendered stress response. Participants were informed they would be asked to complete two challenging tasks. The speech task was described as cognitively challenging and requiring participants to "think on their feet". The SECPT was described as a physically challenging task. Participants were told the approximate length of the tasks. Particular emphasis was given to the participants' right to withdraw from the study at any time before or during study participation and upon hearing the exact nature of the stressful tasks. In an attempt to reduce habituation over stress exposures participants were not told that Stress Visit 2 would very similar to Stress Visit 1, only that it would again comprise a cognitively and physically challenging task. Social-evaluative panel members were informed to observe participants for pronounced negative reactions to the stress protocol and to terminate the procedure if they had any concerns for participants' well-being. Increases in SBP in excess of 180-185 mmHg also resulted in the termination of testing. Participants were falsely told that the speech and SECPT would be video and audio recorded. A partial debrief was given after the completion of Stress Visit 1. The full nature of the study was, however, not revealed until completion of Stress Visit 2 as knowledge of the study and social-evaluative manipulations would likely reduce the perceived stressful nature of the tasks.

The study was approved by the University of Leeds' Institute of Psychological Sciences Research Ethics Committee (Ref: 12-0016; submitted and approved January 2012). An honorarium of £40 was paid to participants upon completion of the study.

6.3.12 Statistical Analyses

Cortisol data (time point measures and AUCg) were skewed and normalized using logarithmic transformations. One participant was unable to return to complete Stress Visit 2. Data for this participant from the control and Stress Visit 1 were retained for analysis. One participant dropped out of the study after completion of the control visit. All data from this participant were removed from analyses. Cortisol results for two participants were removed from Stress Visit 2 due to assayed saliva levels being > 5

SD above sample mean. The presence of blood/foreign bodies in the samples was suspected.

Paired T-tests were employed to compare participant characteristics (reported chronic stress [PSS] and SECPT hand submersion time) across stress visits. The SAS-mixed models procedure (PROC MIXED) was employed to examine the within-subjects change in stress response outcome variables and capillary glucose within and between control and stress visits. Participant ID was entered as a random factor; visit and time were entered as fixed factors. The SAS-mixed models procedure (PROC MIXED) was also employed to examine the within-subjects change in cognitive performance across study visits. Participant ID was entered as a random factor; visit, visit order (order of exposure to counterbalanced control and Stress Visit 1), and trial (task-switch test only) were entered as fixed factors. Age, BMI, PSS scores (Stress Visit 1 and 2) were also initially included as covariates in all models but subsequently removed from due to non-significance in each model.

To explore whether cognitive performance was impaired in high cortisol responders a median split of participants based upon salivary cortisol delta increase at each stress visit was employed. Cognitive performance outcomes for the 50th percentile with the highest response at each stress visit (Stress Visit 1, n = 12; Stress Visit 2, n = 11) were separately compared to performance levels on the control day using paired T-tests.

Partial correlations (two-tailed) were employed to explore the relationship between cortisol responses, and subjective stress ratings (SACL) and cortisol and cardiovascular responses. The statistical approach employed for exploratory regression analyses is described in General Methodologies Section 0). The method of selecting covariance structure for the mixed models is described in General Methodologies Section 3.6.2.

6.4 Results

6.4.1 Participant Characteristics

Participants reported higher chronic stress in the month prior to Stress Visit 2 than the month prior to Stress Visit 1. Paired T-tests revealed significantly higher PSS scores at Stress Visit 2 compared to Stress Visit 1, $t(22) = -4.58$, $p < .001$. Perceived stress

scores at both time points were included as covariates in all analyses of stress response parameters but did not significantly account for any variance in stress or cognitive outcome measures and were subsequently removed from all models. The length of time participants were able to keep their hands submerged in the ice bucket during the SECPT did not differ significantly at Stress Visit 1 or Stress Visit 2, $t(22) = -0.10$, $p = .92$. Participant characteristics are shown in Table 6.2. Participants were free from medication at study entry and reported no use of any medication known to affect HPA axis function between stress visits.

Table 6.2 Participant characteristics (N = 24)

	Age	BMI (kg/m²)	HADS		PSS	
			Anxiety	*Depression*	*Stress visit 1*	*Stress visit 2*
Mean	**21.83**	**22.36**	**2.46**	**3.33**	**8.63**	**12.13**
SEM	0.72	0.37	0.36	0.40	0.81	1.07

HADS - Hospital Anxiety Depression Scale, PSS - Perceived Stress Scale

6.4.2 Salivary Cortisol Response

A significant time×visit interaction, $F(10,215) = 11.93$, $p < .001$, and significant main effects of time, $F(5,115) = 8.65$, $p < .001$, and visit, $F(2,43) = 94.89$, $p < .001$, were revealed for salivary cortisol response (Figure 6.3).

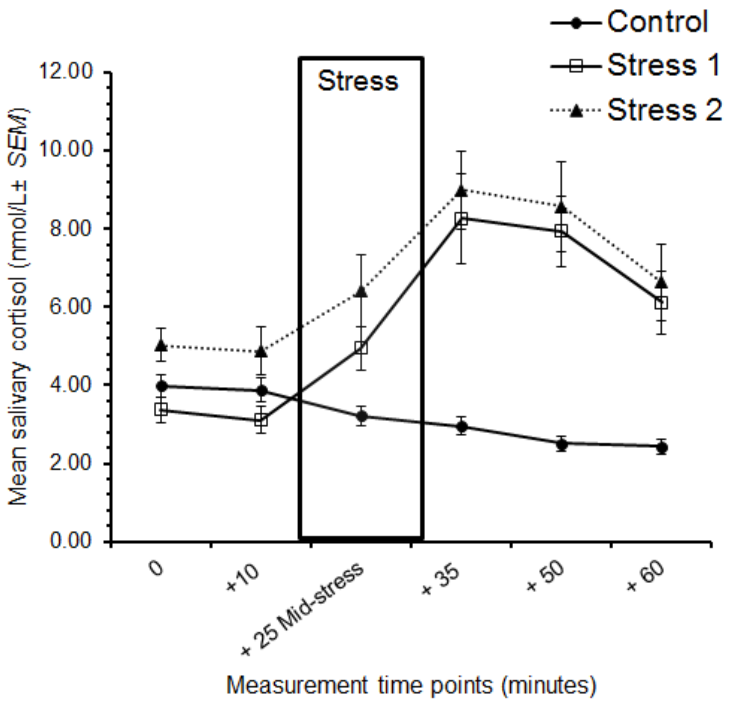


Figure 6.3 Mean (± *SEM)* salivary cortisol response (nmol/L) across control and stress visits

The significant time×visit interaction reflects significantly elevated salivary cortisol responses across both stress visits, whilst a significant decrease was demonstrated on the control visit. Significantly lower levels of salivary cortisol were demonstrated at + 50 and + 60 minutes compared to 0 and + 10 minutes during the control visit (all significant at $p < .03$). During Stress Visit 1 cortisol levels at + 25, + 35, + 50, and + 60 were significantly higher than pre-stress measures at 0 and + 10 minutes (all significant at $p < .05$). Cortisol continued to rise significantly across the response profile with levels at + 35 and + 50 minutes significantly higher than + 25 minutes (all significant at $p < .02$). During Stress Visit 2, peak cortisol levels at + 35 were significantly higher than pre-stress measures at 0 and + 10 minutes (significant at $p < .03$). Cortisol levels at + 50 were also significantly higher than + 10 minutes ($p < .03$).

Mean salivary cortisol levels during Stress Visits 1 and 2 were significantly higher than corresponding control visit levels at + 25, + 35, + 50, and + 60 minutes (all significant at $p < .001$). Interestingly, a significant baseline anticipatory effect was evident at Stress Visit 2. Significantly higher anticipatory pre-stress cortisol levels were evident during Stress Visit 2 than during Stress Visit 1 and control. Cortisol levels at 0 and + 10 minutes were significantly higher than corresponding levels during both the control

visit and Stress Visit 1 (all significant at $p < .03$). However, no significant differences between cortisol levels were revealed between Stress Visits 1 and 2 from mid-stress (+ 25 minutes) onwards.

The aggregated measures of cortisol response across each study visit are shown in Figure 6.4 (AUCi and AUCg) and Figure 6.5 (delta increase). A significant main effect of visit was revealed for AUCi, $F(2,40) = 11.50$, $p < .001$, AUCg, $F(2,40) = 14.01$, $p < .001$, and delta increase, $F(2,40) = 12.06$, $p < .001$. Post-hoc comparisons showed that Stress Visits 1 and 2 provoked significantly higher aggregated cortisol response than the control visit across all aggregated measures (all significant at $p < .003$). A pattern of higher anticipatory cortisol levels at Stress Visit 2 resulted in higher AUCg and lower delta increase and AUCi levels at this visit compared to Stress Visit 1. However, these differences were not significant ($p > .14$).

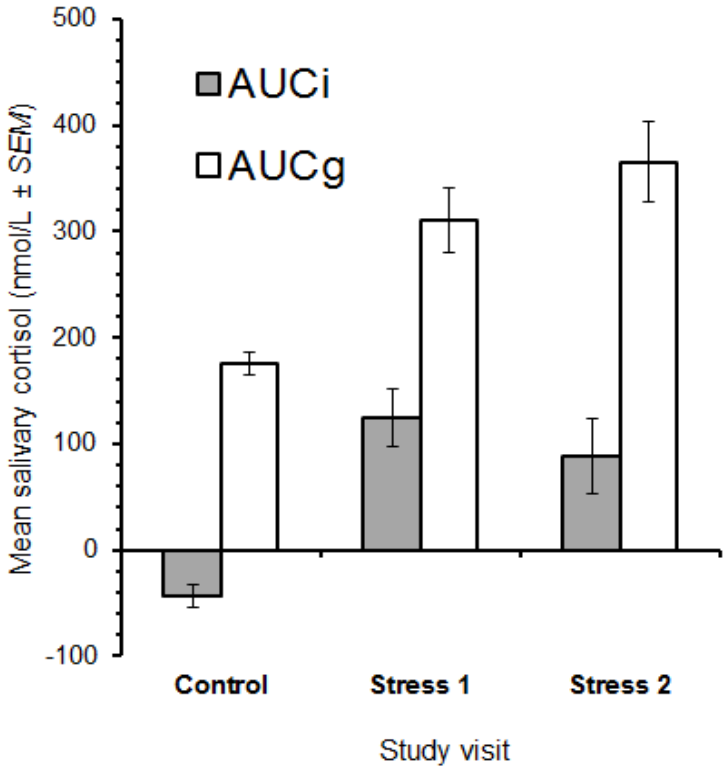


Figure 6.4 Mean (± *SEM)* salivary cortisol AUCg and AUCi (nmol/L) across study visits

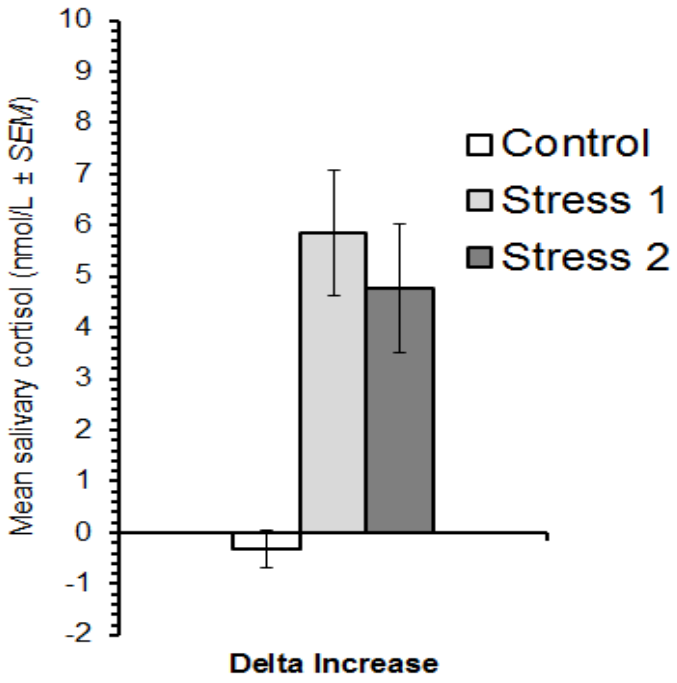


Figure 6.5 Mean (± *SEM)* salivary cortisol delta increase (nmol/L) across study visits

6.4.2.1 Salivary cortisol responses across stress visits

The salivary cortisol response profiles across Stress Visits 1 and 2 were inspected to identify patterns of responses to repeated stress exposure. A fixed threshold classification criterion of a 2.5 nmol/L increase in cortisol has been widely adopted to indicate a distinct secretory cortisol episode (Schommer et al., 2003; Van Cauter & Refetoff, 1985). Participants demonstrating a delta increase response below this threshold across both stress visits were considered non-responders (*n* = 3). Participants were further categorised into 'no change', 'sensitisation', 'habituation', groups based upon their responses over the two stress visits. The grouping of participants was based upon inspection of their cortisol response profiles. Participants categorised as no change demonstrated comparable response profiles across both study visits. A sensitisation in response was characterised by heightened responses at Stress Visit 2 (anticipatory or post-stress), whilst habituating participants were characterised by lower peak responses at Stress Visit 2. The cortisol response profiles and delta increase for each response type are shown in Figure 6.6 for illustrative purposes. These data are based on 21 participants since no Stress Visit 2 data was available for 3 participants owing to withdrawal (*n* = 1) and salivary cortisol > 5 SD above the sample mean (*n* = 2).

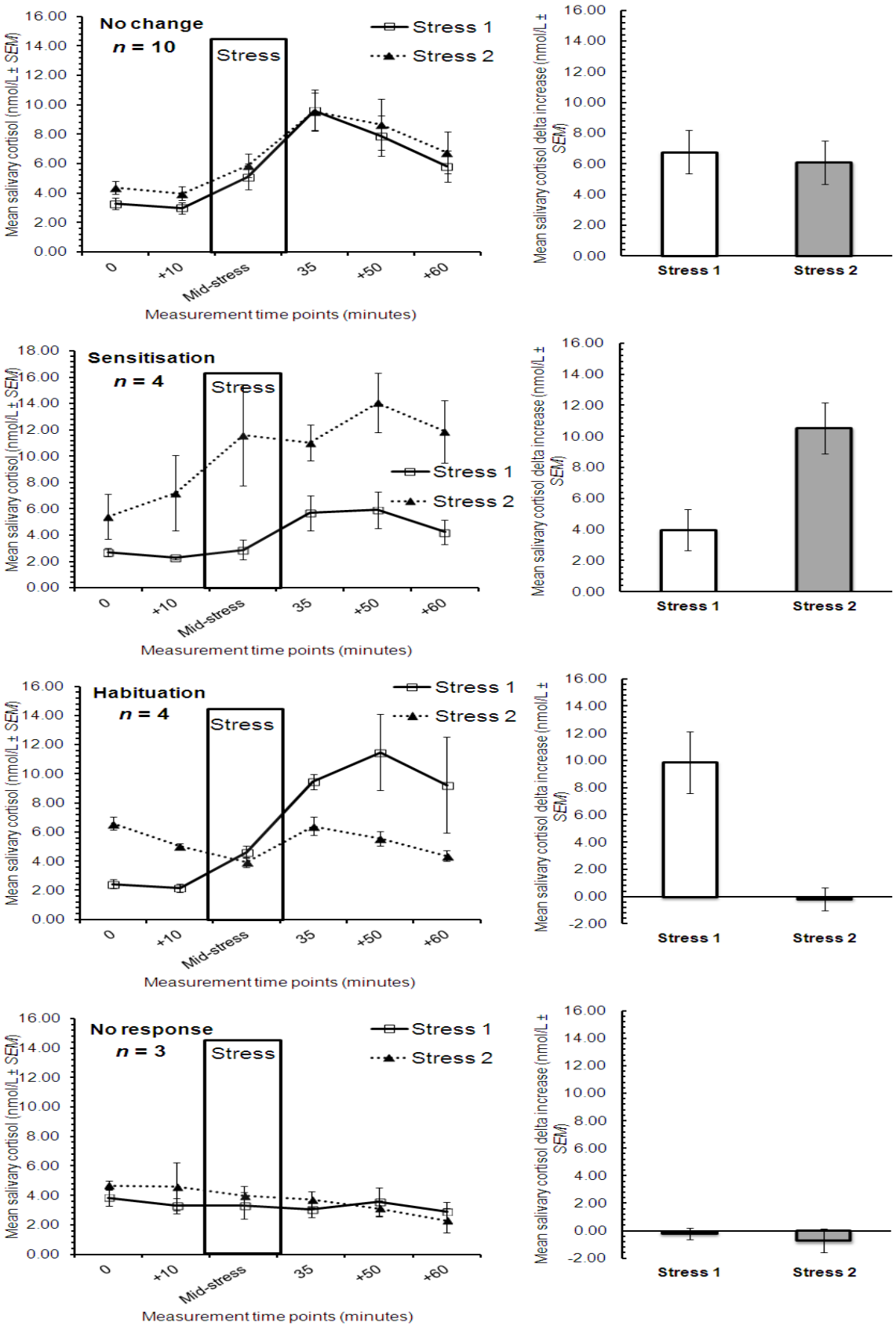


Figure 6.6 Mean (± *SEM)* salivary cortisol response profile (left panel) and delta increase (nmol/L; right panel) across habituation type

6.4.3 Cardiovascular Response

6.4.3.1 Systolic blood pressure

A significant time×visit interaction, $F(14,314) = 11.06$, $p < .001$, and significant main effects of time, $F(8,161) = 58.44$, $p < .001$, and visit, , $F(2,45) = 97.83$, $p < .001$, were revealed for SBP response (see Figure 6.7).

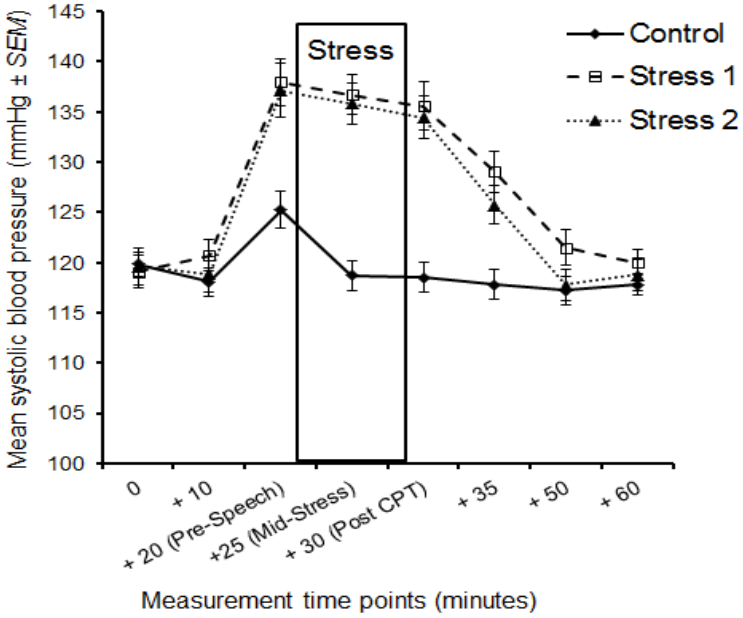


Figure 6.7 Mean (± *SEM)* SBP (mmHg) across control and stress visits

The significant time×visit interaction reflects significant increases in SBP across both stress visits, whilst only a minor excursion from baseline levels was demonstrated during the control visit. The increase in SBP during the control condition (SBP at + 20 minutes was significantly higher than all other measures (all significant at $p < .03$). This corresponds to the time point at which participants were asked to walk to the stress test room (see Section 6.3.10.1).

During both stress visits, SBP at + 20, + 25, + 30, and + 35 was significantly higher than pre-stress measures at 0 and + 10 minutes (all significant at $p < .03$). Systolic BP had significantly lowered by 5 minutes post-stress with SBP at + 20, + 25 and + 30 higher than measures at + 35, + 50 and + 60 minutes (all significant at $p < 0.03$).

Mean SBP during Stress Visits 1 and 2 were significantly higher than corresponding control levels at + 20, +25, + 30, and + 35 minutes (all significant at $p < .001$). No significant differences in SBP were revealed between Stress Visits 1 and 2.

6.4.3.2 Diastolic blood pressure

A time×visit interaction, $F(14,315) = 6.32$, $p < .001$, and significant main effects of time, $F(7,161) = 27.51$, $p < .001$, and visit, $F(2,45) = 45.23$, $p < .001$, were revealed for DBP response (Figure 6.8).

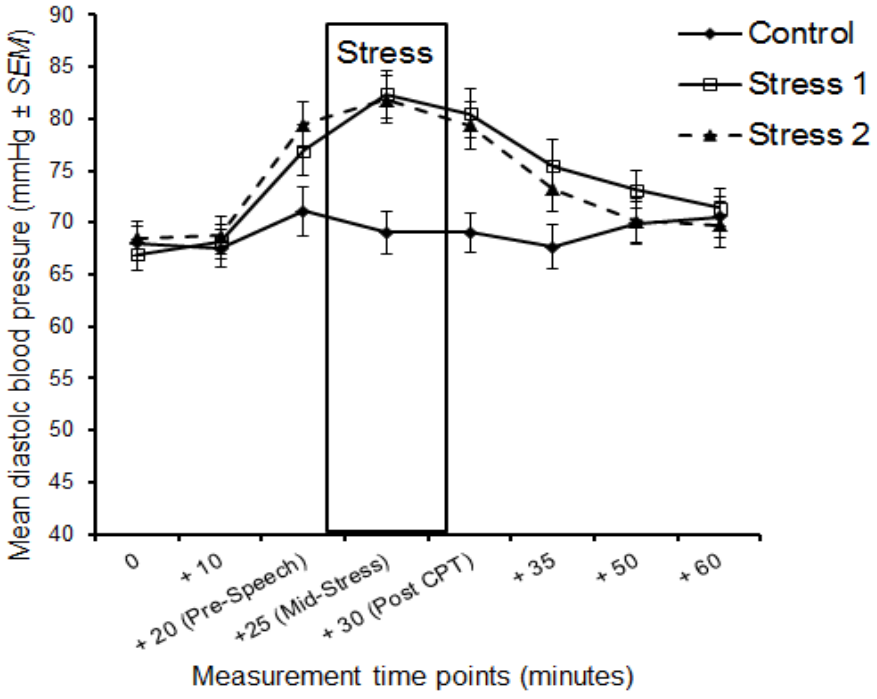


Figure 6.8 Mean (± *SEM)* DBP (mmHg) across control and stress visits

The significant time×visit interaction reflects significant increases in DBP across both stress visits, whilst no significant differences in DBP response were demonstrated during the control visit.

During both stress visits, DBP at + 20, + 25, + 30, and + 35 minutes was significantly higher than pre-stress measures at 0 and + 10 minutes (all significant at $p < .02$). Diastolic BP was also significantly higher mid-stress (+ 25 minutes) than post-stress measures at + 35, + 50, and + 60 minutes, and post-stress + 30 compared to + 50 and + 60 minutes (all significant at $p < .03$),

Mean DBP during Stress Visit 1 was significantly higher than corresponding control levels at + 25, + 30, and + 35 minutes (all significant at $p < .001$). Mean DBP during visit 2 was significantly higher than corresponding control levels at + 20, + 25, and + 30 minutes (all significant at $p < .001$). No significant differences between DBP were revealed between Stress Visits 1 and 2.

6.4.3.3 Heart rate

A significant time×visit interaction, $F(14,315) = 2.40$, $p < .001$, and significant main effects of time, $F(7,161) = 3.40$, $p < .001$, and visit, $F(2,45) = 32.19$, $p < .001$, were revealed for HR response (Figure 6.9).

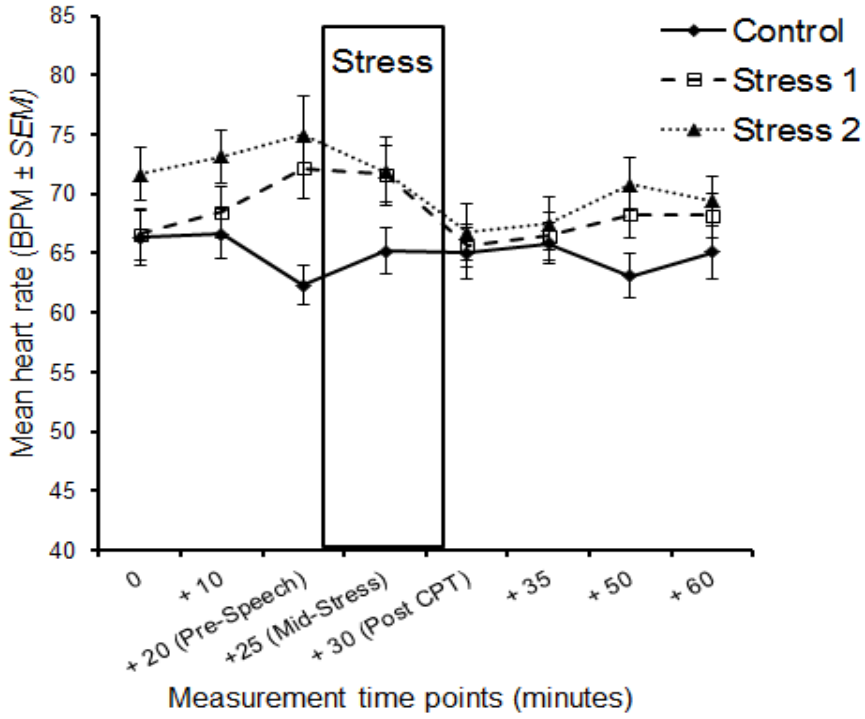


Figure 6.9 Mean (± *SEM)* HR (bpm) across control and stress visits

The significant time×visit interaction reflects no significant increases in heart rate across the response profiles during control and Stress Visit 1. Anticipatory pre-stress HR response was significantly higher at + 20 compared to post-stress levels at + 30 and + 35 minutes during Stress Visit 2 (both significant at $p < .04$).

Comparison of HR response between visits revealed significantly higher HR at + 20 and + 25 minutes during Stress Visit 1 and Stress Visit 2 compared to control (all

significant at p < .03). Participants demonstrated a significantly higher mean HR response at + 50 minutes during Stress Visit 2 compared to the corresponding control measure. No significant differences between the two stress conditions were revealed.

6.4.4 Subjective Responses

6.4.4.1 Subjective stress (SACL)

A significant time×visit interaction, $F(8,177) = 3.81$, $p < .001$, and significant main effects of time, $F(4,92) = 2.41$, $p = .05$, and visit, $F(2,45) = 20.13$, $p < .001$, were revealed for subjective stress ratings (see Figure 6.10)

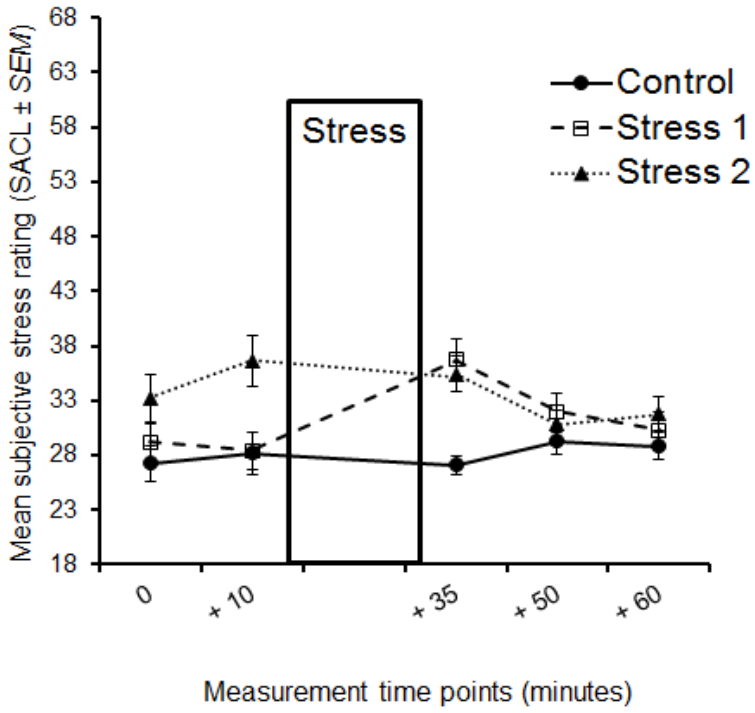


Figure 6.10 Mean (± *SEM)* subjective stress ratings (SACL) across control and stress visits

The significant time×visit interaction reflects different stress response profiles across the control and stress visits. No significant differences were revealed across the control visit response profile. During Stress Visit 1 subjective stress rating was significantly higher post-stress at + 35 minutes compared to pre-stress at 0 and + 10 minutes (both significant at $p < .01$). An anticipatory baseline subjective stress

response at Stress Visit 2 resulted in no significant increases in stress ratings across the profile. However, stress ratings across both stress visits were significantly higher at + 35 minutes compared to the control visit. An anticipatory peak subjective stress rating at +10 minutes during Stress Visit 2 was significantly higher than both corresponding ratings at control and Stress 1 visits (both significant at p <.001).

6.4.4.2 Subjective arousal (SACL)

A significant time×visit interaction, $F(8,177) = 3.42$, $p < .001$, and a main effect of time, $F(4,92) = 2.84$, $p < .03$, were revealed for subjective arousal (see 6.11).

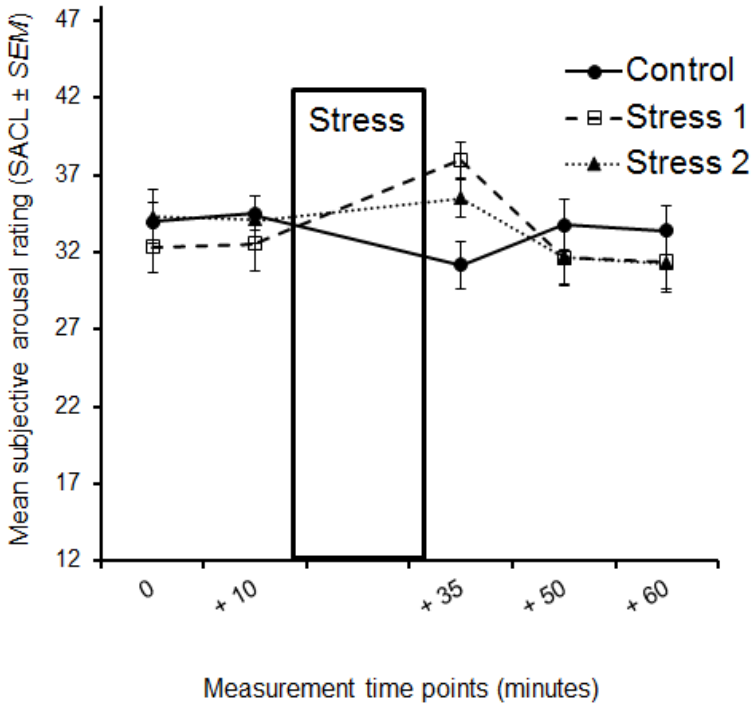


Figure 6.11 Mean (± *SEM)* subjective arousal ratings (SACL) across control and stress visits

The significant time×visit interaction reflects different stress response profiles across the control and stress visits. No significant differences were revealed across the Stress Visit 2 and control visit profile. Subjective arousal ratings during Stress Visit 1 peaked post-stress at + 35. This mean arousal rating was significantly higher than pre-stress measures at 0 and +10 minutes, and post-stress measures at + 50 and + 60 minutes. This Stress Visit 1 rating was also significantly higher than the mean

corresponding + 35 minutes arousal ratings during the control visit (all significant at $p < .001$). No differences between arousal rating at Stress Visit 2 and control reached significance.

6.4.4.3 Profile of Mood States

All *means* and *SEM* of subjective POMS dimension mood ratings are shown in Table 6.3. No significant effects of Anger-hostility were revealed.

6.4.4.3.1 Tension-anxiety

A significant time×visit interaction, $F(8,177) = 2.65$, $p < .001$, and main effects of time, $F(4,92) = 2.52$, $p = .05$, and visit, $F(2,45) = 11.53$, $p < .001$, were revealed for subjective Tension-anxiety ratings. Post-hoc comparisons revealed ratings of Tension-anxiety to be significantly higher during Stress Visit 1 at + 35 minutes compared to control. Higher baseline ratings (0 and + 10 minutes) at Stress Visit 2 compared to control and Stress Visit 1 were apparent (see Table 6.3) but these differences did not reach significance ($p = .10$).

6.4.4.3.2 Confusion-bewilderment

A significant main effect of visit, $F(2,45) = 15.20$, $p < .001$, was revealed for subjective Confusion-bewilderment ratings. Participants reported significantly higher confusion after stress exposure (+ 35 minutes) during Stress Visit 1 compared to the control visit ($p = .02$).

6.4.4.3.3 Depression-dejection

A significant main effect of visit, $F(2,45) = 13.38$, $p < .001$, was revealed for subjective Depression-dejection ratings. Post-hoc comparisons revealed no significant differences within or between study visits.

6.4.4.3.4 Vigour-activity

A significant time×visit interaction, $F(8,177) = 4.98$, $p < .001$, and main effect of time, $F(4,92) = 4.98$, $p < .001$, were revealed for subjective Vigour-activity ratings. Post-hoc comparisons revealed no significant differences within or between study visits.

6.4.4.3.5 Fatigue-inertia

Significant main effects of time, $F(4,92) = 4.56$, $p < .001$, and visit, $F(2,45) = 5.82$, $p < .001$, were revealed for subjective Vigour-activity ratings. Post-hoc comparisons revealed no significant differences within or between study visits.

6.4.4.3.6 Depression-dejection

A significant main effect of visit, $F(2,45) = 13.38$, $p < .001$, was revealed for subjective Depression-dejection ratings. Post-hoc comparisons revealed no significant differences within or between study visits.

6.4.4.3.7 Vigour-activity

A significant time×visit interaction, $F(8,177) = 4.98$, $p < .001$, and main effect of time, $F(4,92) = 4.98$, $p < .001$, were revealed for subjective Vigour-activity ratings. Post-hoc comparisons revealed no significant differences within or between study visits.

6.4.4.3.8 TMD

A significant main effect of visit, $F(2,45) = 12.81$, $p < .001$, and time, $F(4,92) = 3.53$, $p < .01$, was revealed for TMD score. However, post-hoc comparisons revealed no significant differences within or between study visits.

Table 6.3 Mean (± *SEM)* subjective mood ratings (POMS) across control and stress visits. Score ranges are shown in parentheses next to each dimension

	Study Visit	Time in minutes relative to audition onset									
		0		+ 10		+ 35		+ 50		+ 60	
		Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
POMS											
	Control	**2.13**	0.68	**2.17**	0.76	**1.08**	0.44	**1.88**	0.58	**1.46**	0.46
Tension-anxiety (0 - 24)	Stress 1	**2.21**	0.63	**2.42**	0.73	**4.17**	0.80	**1.92**	0.57	**2.25**	0.73
	Stress 2	**4.32**	1.00	**4.36**	0.97	**2.86**	0.83	**2.39**	0.74	**2.48**	0.83
	Control	**1.79**	0.32	**1.63**	0.31	**1.58**	0.20	**1.50**	0.28	**1.71**	0.27
Depression-dejection (0 - 32)	Stress 1	**2.42**	0.58	**2.33**	0.51	**2.25**	0.36	**2.75**	0.53	**2.71**	0.49
	Stress 2	**3.18**	0.70	**3.27**	0.73	**2.86**	0.52	**2.78**	0.54	**2.57**	0.45
	Control	**0.13**	0.07	**0.17**	0.12	**0.29**	0.18	**0.63**	0.36	**0.46**	0.26
Anger-hostility (0 - 28)	Stress 1	**0.96**	0.50	**0.83**	0.38	**1.00**	0.43	**1.25**	0.72	**1.42**	0.66
	Stress 2	**1.50**	0.69	**1.82**	0.96	**0.95**	0.48	**1.52**	0.63	**1.48**	0.62
	Control	**7.50**	0.82	**6.13**	0.84	**4.75**	0.74	**4.79**	0.79	**5.33**	0.76
Vigour-activity (0 - 24)	Stress 1	**6.54**	1.11	**5.54**	0.90	**7.17**	0.93	**5.00**	0.84	**5.21**	0.87
	Stress 2	**6.91**	1.07	**6.27**	0.94	**7.05**	0.86	**5.04**	0.93	**5.74**	0.99
	Control	**1.17**	0.27	**0.50**	0.16	**1.71**	0.43	**2.00**	0.65	**1.58**	0.45
Fatigue-inertia (0 - 20)	Stress 1	**1.88**	0.59	**1.83**	0.49	**1.25**	0.38	**2.75**	0.71	**3.21**	0.78
	Stress 2	**2.18**	0.85	**1.45**	0.62	**1.18**	0.44	**2.74**	0.68	**2.78**	0.76
	Control	**1.21**	0.34	**0.96**	0.29	**0.67**	0.23	**0.92**	0.38	**0.88**	0.20
Confusion-bewilderment (0 - 20)	Stress 1	**2.08**	0.46	**1.88**	0.51	**2.17**	0.44	**1.54**	0.45	**1.63**	0.50
	Stress 2	**1.91**	0.54	**1.68**	0.50	**1.09**	0.42	**1.39**	0.48	**1.57**	0.49
	Control	**6.41**	1.22	**5.42**	1.20	**5.33**	0.93	**6.92**	1.43	**6.08**	0.96
TMD (0 - 124)	Stress 1	**9.54**	2.07	**9.29**	1.89	**6.67**	1.25	**12.46**	2.29	**10.87**	2.54
	Stress 2	**12.52**	3.29	**12.04**	3.26	**5.83**	1.58	**11.17**	2.33	**10.78**	2.50

POMS = Profile of Mood States, TMD = Total Mood Disturbance

Highlighted sections denote significant differences between study visits

6.4.5 Subjective Stress and Physiological Stress Response

Pearson's Product Moment (two-tailed) correlations revealed a significant relationship between salivary cortisol and subjective stress rating at + 60 minutes, $r(21) = .45$, $p = .03$, and subjective stress and diastolic BP at + 35 minutes, $r(21) = .41$, $p = .05$, during Stress Visit 1. No further significant correlations emerged.

6.4.6 Cognitive Performance

6.4.6.1 VISGED

A one-way ANOVA revealed no significant differences between the time taken to complete the VISGED across control and stress visits, $F(2,70) = 0.53$, $p = .60$. There was no significant main effect of visit, $F(2,45) = 2.46$, $p = .10$, for VISGED visual memory score (Figure 6.12).

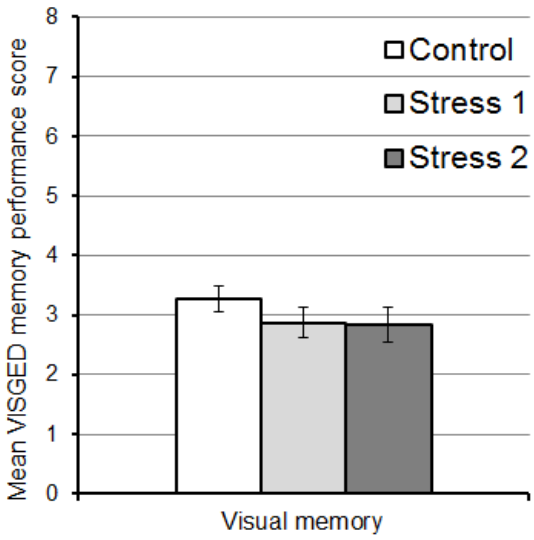


Figure 6.12 Mean (± *SEM)* VISGED visual memory performance across control and stress visits

6.4.6.2 2-back

Non-significant trends for effects of visit on target RT, $F(2,45) = 2.58$, $p = .08$, and non-target RT, $F(2,45) = 2.47$, $p = .08$, were revealed (Figure 6.13). Descriptive trends towards faster RT responses to target and non-target stimuli during Stress Visit 1 compared to control were demonstrated ($p = .09$). No significant main effects of visit

were revealed for 2-back target accuracy, $F(2,45) = 1.12$, $p = .33$, or total accuracy, $F(2,45) = 1.25$, $p = .30$ (Figure 6.14).

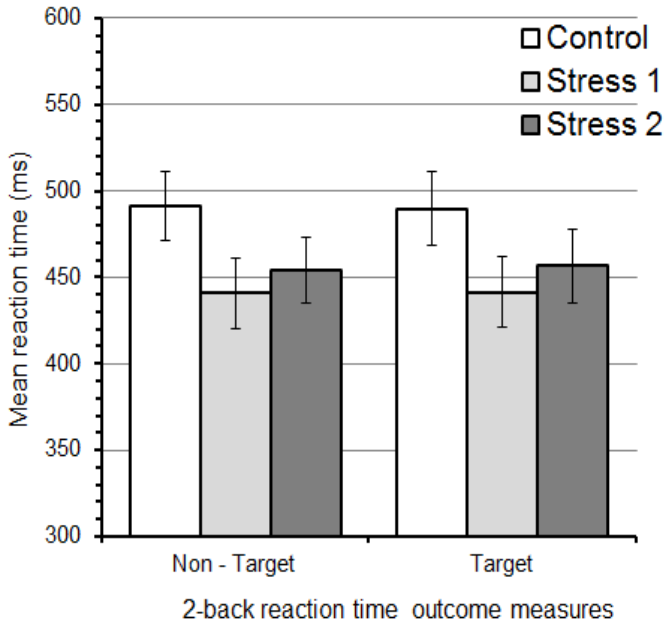


Figure 6.13 Mean (± *SEM)* 2-back target and non-target RT (ms) across control and stress visits

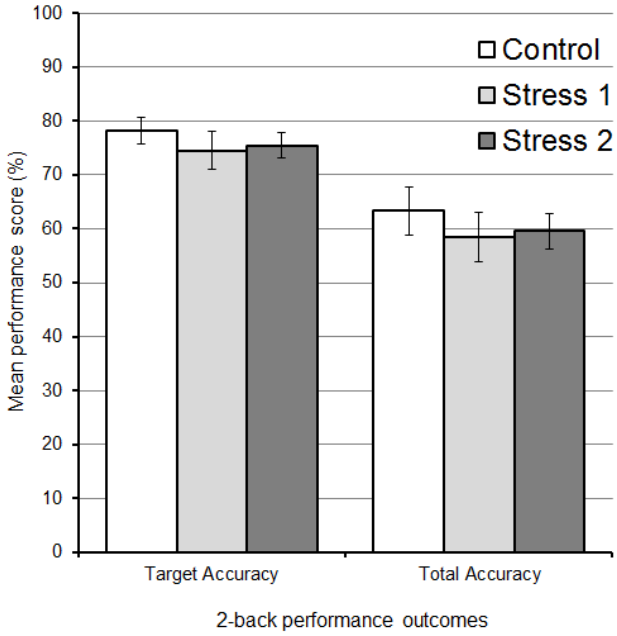


Figure 6.14 Mean (± *SEM)* 2-back target and total accuracy (%) across control and stress visits

6.4.6.3 A-ospan

A significant main effect of visit on A-ospan total, $F(2,45) = 4.00$, $p = .03$, and non-significant trend of visit on A-ospan score, $F(2,45) = 2.56$, $p = .09$, were revealed. Post-hoc comparisons revealed a non-significant trend for impaired A-ospan total during Stress Visit 1 and Stress Visit 2 compared to control (both significant at $p = .07$; Figure 6.15). There were no significant main effects of visit for A-ospan accuracy error, $F(2,45) = 2.18$, $p = .13$,or speed error, $F(2,45) = 0.66$, $p = .52$. Speed error is not shown in the Figure 6.15 due to *mean* speed errors across the study visits being < 1.

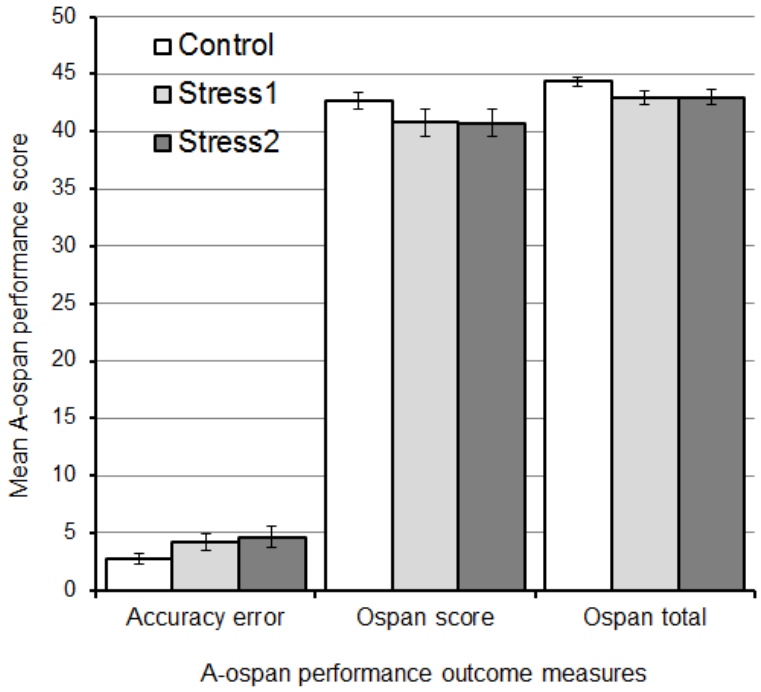


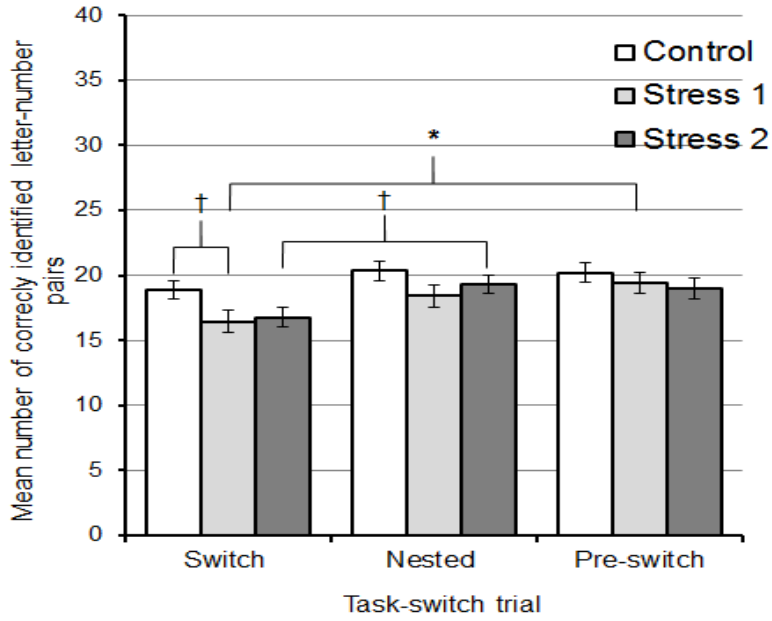
Figure 6.15 Mean (± *SEM)* A-ospan performance outcome measures across control and stress visits

6.4.6.4 Task-switch test

Significant main effects of visit, $F(2,45) = 7.68$, $p < .001$, and trial (switch, nested, and pre-switch), $F(2,46) = 12.95$, $p < .001$, but no significant visit×trial interaction, $F(4,90) = 0.70$, $p = .59$, were revealed for trial accuracy (number of correctly identified letter-number pairs).

Post-hoc comparisons revealed no significant differences between the numbers of correctly identified target letter-number pair targets across trial type during the control

condition. During Stress Visit 1, participants identified significantly fewer targets during the switch trials compared to pre-switch trials (p = .01). A non-significant trend towards fewer correctly identified switch trial targets during Stress Visit 1 compared to control was also demonstrated (p = .07). During Stress Visit 2 a non-significant trend for fewer targets identified during switch trials compared to nested trials was revealed (p = .07; Figure 6.16).



* p = .01, † p = .07

Figure 6.16 Mean (± *SEM)* number of correctly identified letter-number pairs across task-switch trials during control and stress visits

Analysis revealed a significant main effect of trial, $F(2,45) = 37.70$, $p < .001$, but no effect of visit, $F(2,46) = 1.04$, $p = .36$, or visit×trial interaction, $F(4,90) = 0.29$, $p = .88$, for trial RT. Post-hoc tests revealed RTs (ms) for the identification of target letter-number pair targets during switch trials were significantly slower than for repeated nested and pre-switch trials (Figure 6.17). This was consistent across control (p < .001), Stress Visit 1 (p < .001), and Stress Visit 2 (p = .05).

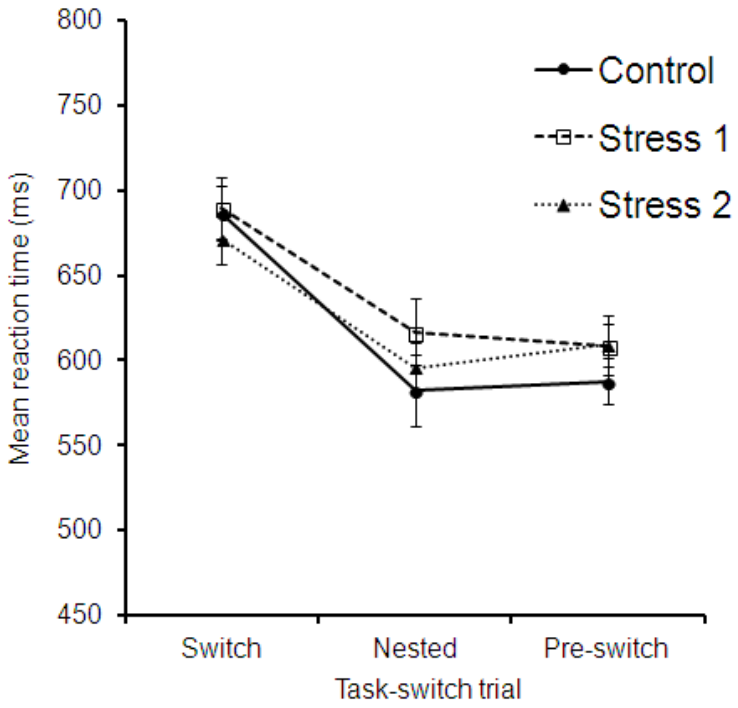
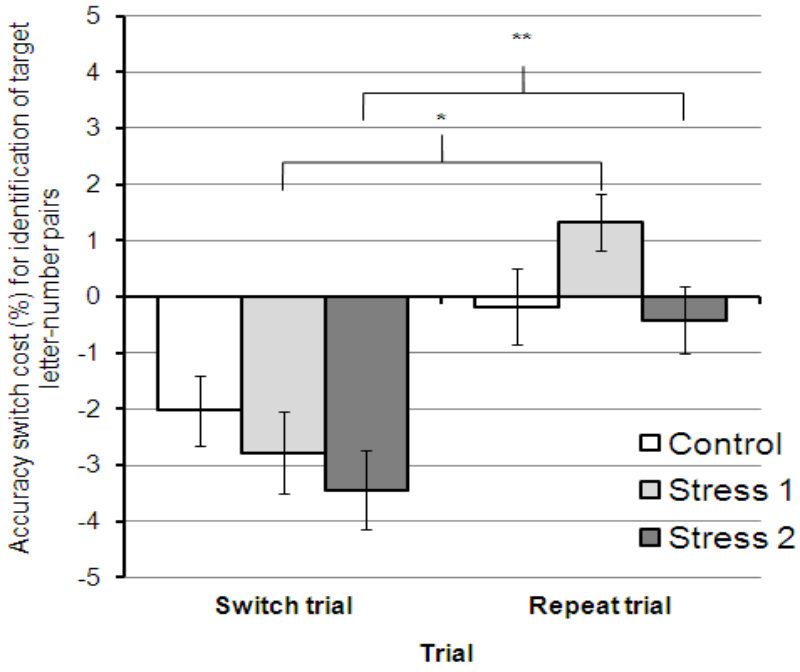


Figure 6.17 Mean (± *SEM)* RT (ms) across task-switch trials during control and stress visits

6.4.6.5 Task-switch test switch costs

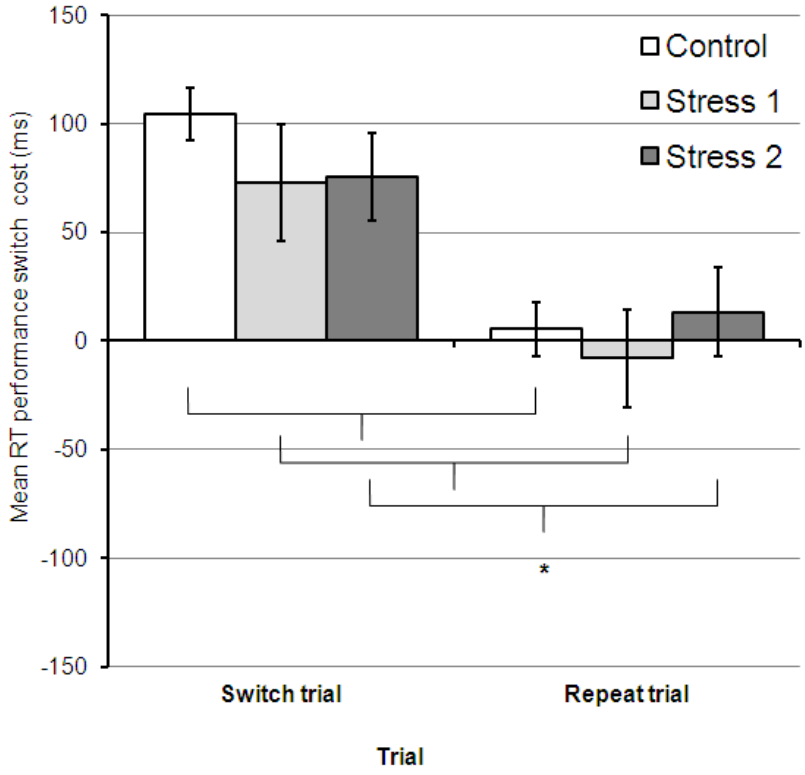
A significant main effect of trial (switch/repeat), $F(1,23) = 31.11$, $p < .001$, but no significant effect of visit, $F(2,45) = 1.69$, $p = .19$, or visit×trial interaction, $F(2,45) = 1.50$, $p = .24$, were revealed for accuracy performance switch cost (Figure 6.18). Post-hoc comparisons revealed that the accuracy switch cost (% performance decrement) incurred during switch trials was significantly higher than the switch cost incurred during repeat trials during both stress visits ($p < .03$) but not during the control visit ($p = .10$).



* $p = .001$; $p = .03$

Figure 6.18 Mean (± *SEM)* performance accuracy (%) switch cost across control and stress visits

Analysis revealed a significant main effect of trial, $F(1,23) = 35.69$, $p < .001$, but no significant effect of visit, $F(2,45) = 0.61$, $p = .55$, or visit×trial interaction, $F(2,45) = 0.27$, $p = .76$, for RT performance switch cost (Figure 6.19). Post-hoc comparisons revealed that the RT switch cost (performance decrement) incurred during switch trials was significantly higher than the switch cost incurred during repeat trials across control and stress visits (all significant at $p < .04$).



* $p < .04$

Figure 6.19 Mean (± *SEM)* performance RT switch cost (ms) across control and stress visits

6.4.6.6 Cognitive performance in high cortisol responders.

Paired T-tests revealed 2-back target accuracy, $t(11) = 2.88$, $p = .02$, and total accuracy, $t(11) = 3.30$, $p = .01$, were both significantly lower during Stress Visit 1 compared to the non-stress control visit in high cortisol responders. A-ospan score, $t(11) = 2.67$, $p = .02$, and total, $t(11) = 2.42$, $p = .03$, were also significantly more impaired during Stress Visit 1 vs. control (see Figure 6.20).

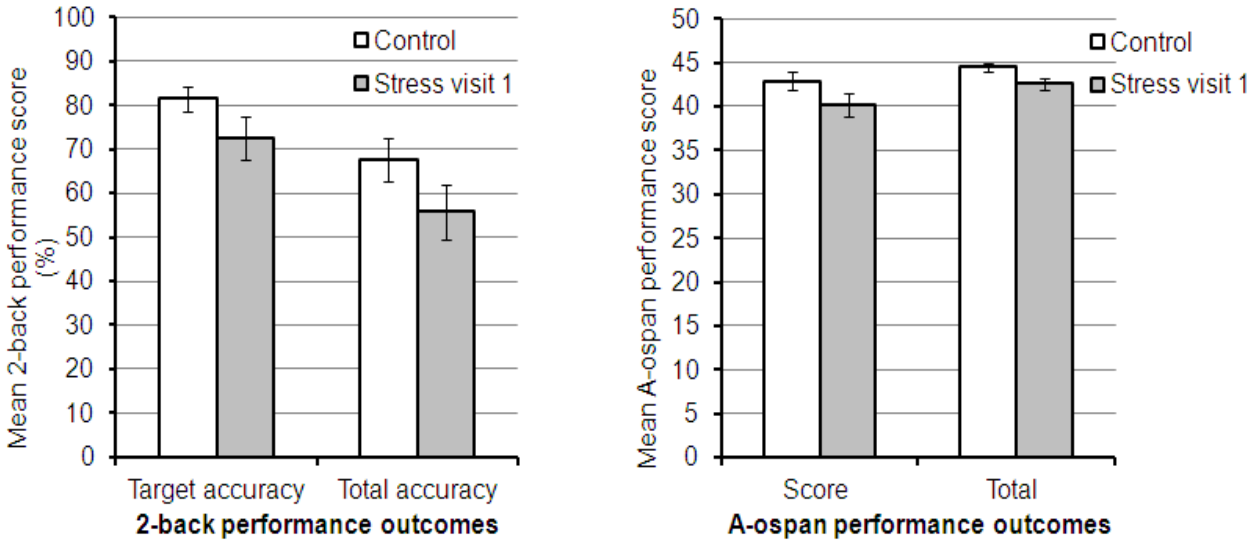


Figure 6.20 Mean (± *SEM)* 2-back target and total accuracy (%) and A-ospan score and total in high cortisol responders (median Stress Visit 1 cortisol delta increase split) at Stress Visit 1 vs. control

During Stress Visit 2, A-ospan total was significantly lower than control visit performance, $t(10) = 2.46$, $p = .03$, and A-ospan score was marginally significantly lower, $t(10) = 2.08$, $p = .06$ (see Figure 6.21).

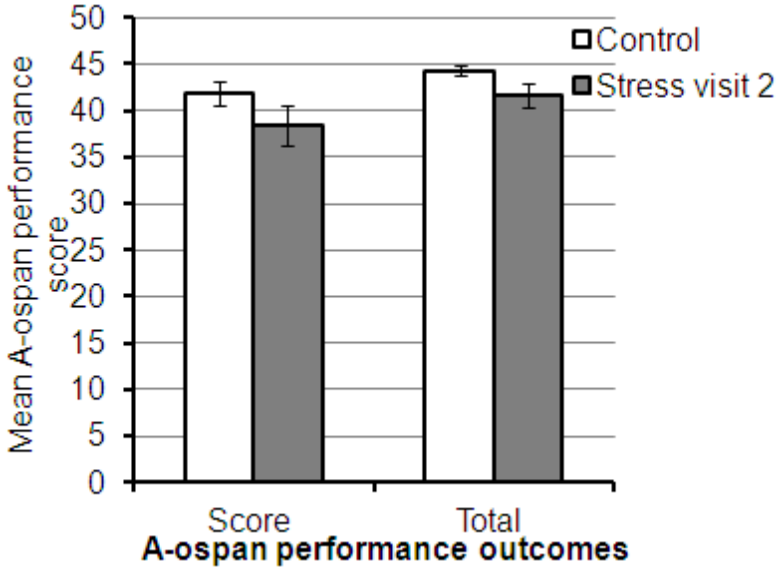


Figure 6.21 Mean (± *SEM)* A-ospan score and total in high cortisol responders (median Stress Visit 2 cortisol delta increase split) at Stress Visit 2 vs. control

6.4.7 Predictors of Cortisol Responsivity

6.4.7.1 Associations between trait variables and cortisol response

6.4.7.1.1 Stress Visit 1

Partial correlations (two-tailed) controlling for age and BMI revealed significant relationships between salivary cortisol AUCg and Perfectionism: Personal Standards, $r(20) = .44$, $p = .04$, Perfectionism: Organisation, $r(20) = .45$, $p = .04$, and Perfectionism Total, $r(20) = .43$, $p = .05$. Salivary cortisol AUCi was significantly related to Perfectionism: Personal Standards, $r(20) = .44$, $p = .04$, and Perfectionism: Organisation, $r(20) = .55$, $p = .008$. Salivary cortisol delta increase was significantly related to Perfectionism: Personal Standards, $r(20) = .50$, $p = .02$, Perfectionism: Organisation, $r(20) = .58$, $p = .005$, and Perfectionism Total, $r(20) = .45$, $p = .03$. Perfectionism: Organisation was significantly related to salivary cortisol levels at + 35, $r(20) = .44$, $p = .04$, + 50, $r(20) = .50$, $p = .02$, and + 60, $r(20) = .46$, $p = .03$, minutes relative to visit commencement. All trait variables associated with a measure of cortisol response were included in each regression model. As Perfectionism: Organisation has emerged as the only significant trait predictor of cortisol response over previous studies, this variable was entered at the second step. All remaining potential predictor trait variables were entered in the third step.

Exploratory regression analysis revealed the control variables (age, BMI, and PSS [Stress Visit 1]) did not significantly predict AUCg, AUCi, or delta increase (step one). The inclusion of Perfectionism: Organisation at step two resulted in models that accounted for 24% of the adjusted variance in AUCg, 34% of the adjusted variance in AUCi, and 33% of the adjusted variance in delta increase (see Table 6.4 for regression model values for aggregated cortisol measures). The addition of Perfectionism: Organisation increased the explained adjusted variance by 9% (AUCg), 19% (AUCi), and 32% (delta increase) suggesting a positive relationship between this domain of perfectionism and cortisol responsivity.

Whilst the model for AUCg at step two was significant, no single variable emerged as a significant predictor. For models of AUCi and delta increase, Perfectionism: Organisation emerged as the only significant predictor. The addition of Perfectionism: Personal Standards and Perfectionism Total at step three resulted in non-significant

models for AUCg and AUCi and a reduction in the explained variance of 4% and 4.8% respectively. The model for cortisol delta increase remained significant after the inclusion of Perfectionism: Personal Standards and Perfectionism Total at step three (p = .05) but the explained variance was reduced by 3%. Perfectionism: Organisation emerged as a marginal predictor in this model (p =.07).

Exploratory regression analysis resulted in non-significant regression models for salivary cortisol measures at + 35, + 50, or + 60 minutes relative to visit commencement (see Table 6.5 for regression model values for cortisol measures).

Table 6.4 Hierarchical multiple regression analyses of relationships between Perfectionism: Organisation, Personal Standards, and Total, and aggregated measures of salivary cortisol (controlling for age, BMI and PSS [Stress Visit 1])

		AUCg					*AUCi*					*Delta Increase*			
		B	*SE B*	*β*	*F* change		*B*	*SE B*	*β*	*F* change		*B*	*SE B*	*β*	*F* change
Step 1															
	Constant	1.82	0.69			Constant	186.18	251.93			Constant	11.51	21.02		
	Age	0.03	0.01	.45		Age	14.78	8.48	.38		Age	0.45	0.39	.27	
	BMI	0.01	0.03	.08		BMI	-12.89	18.06	-.18		BMI	-0.47	0.84	-.14	
	PSS	-0.03	0.01	-.50	2.38	PSS	-11.16	9.02	-.33	2.32	PSS	-0.58	0.42	-.39	1.84
Step 2															
	Constant	1.98	0.66			Constant	324.28	200.04			Constant	18.27	18.23		
	Age	0.02	0.01	.39		Age	8.26	7.84	.22		Age	0.14	0.36	.08	
	BMI	-0.001	0.03	-.09		BMI	-21.86	16.22	-.30		BMI	-0.91	0.74	-.27	
	PSS	-0.02	0.01	-.38		PSS	-5.43	8.21	-.16		PSS	-0.30	0.37	-.20	
	Perfectionism Org	-0.01	0.01	.36	3.31	Perfectionism Org	10.47	3.97	.47*	6.97*	Perfectionism Org	0.51	0.18	.51*	8.04*
Step 3															
	Constant	1.54	0.79			Constant	111.57	286.00			Constant	5.77	21.85		
	Age	0.02	0.01	.42		Age	9.60	8.71	.26		Age	0.19	0.39	.11	
	BMI	0.01	0.28	.04		BMI	-18.69	17.30	-.25		BMI	-0.74	0.78	-.22	
	PSS	-0.02	0.02	-.36		PSS	-5.75	9.90	-.17		PSS	-0.28	0.45	-.18	
	Perfectionism Org	0.01	0.01	.34		Perfectionism Org	10.87	5.84	.49		Perfectionism Org	0.50	0.26	.50	
	Perfectionism PS	-0.01	0.02	-.14		Perfectionism PS	-4.56	10.63	-.15		Perfectionism PS	-0.18	0.48	-.13	
	Perfectionism Total	0.004	0.004	.27	0.54	Perfectionism Total	1.94	2.30	.21	0.36	Perfectionism Total	0.11	0.10	.27	0.60

AUCg Step 1: $R^2 = .26$, $\Delta R^2 = .15$, $F(3,23) = 2.28$, $p = .10$; Step 2: $R^2 = .37$, $\Delta R^2 = .24$, $F(4,23) = 2.82$, $p = .05$, Step 3: $R^2 = 41$, $\Delta R^2 = .20$, $F(6,23) = 1.97$, p = .13

AUCi Step 1: $R^2 = .26$, $\Delta R^2 = .15$, $F(3,23) = 2.32$, $p = .11$; Step 2: $R^2 = .46$, $\Delta R^2 = .34$, $F(4,23) = 4.00$, $p = .01$, Step 3: $R^2 = 47$, $\Delta R^2 = .30$, $F(6,23) = 2.61$, p = .06

Delta Increase Step 1: $R^2 = .22$, $\Delta R^2 = .09$, $F(3,23) = 1.84$, $p = .17$; Step 2: $R^2 = .45$, $\Delta R^2 = .33$, $F(4,23) = 3.88$, $p = .02$, Step 3: $R^2 = 48$, $\Delta R^2 = .30$, $F(6,23) = 2.68$, p = .05

* $p < .01$

Perfectionism PS - Personal Standards, Perfectionism Org - Organisation

Table 6.5 Hierarchical multiple regression analyses of relationships between Perfectionism: Organisation and Personal Standards, and salivary cortisol measures (controlling for age, BMI and PSS [Stress Visit 1])

	Measurement time point (relative to visit onset)											
	+ 35				+ 50				+ 60			
		B	*SE B*	*β*	*F* change		*B*	*SE B*	*β*	*F* change		*B*	*SE B*	*β*	*F* change
Step 1															
	Constant	0.46	0.98			Constant	0.65	0.92			Constant	0.56	0.89		
	Age	0.30	0.02	.38		Age	0.03	0.02	.38		Age	0.03	0.02	.42	
	BMI	-0.001	0.04	.01		BMI	-0.10	0.04	-.07		BMI	-0.01	0.04	-.10	
	PSS	-0.03	0.02	-.44	1.68	PSS	-0.02	0.02	-.34	1.54	PSS	-0.02	0.02	-.35	1.89
Step 2															
	Constant	0.69	0.93			Constant	0.89	0.85			Constant	0.78	0.83		
	Age	0.02	0.02	.25		Age	0.02	0.02	.22		Age	0.02	0.02	.28	
	BMI	-0.02	0.04	-.10		BMI	-0.03	0.03	-.18		BMI	-0.28	0.03	.20	
	PSS	-0.02	0.02	-.31		PSS	-0.01	0.02	-.19		PSS	-0.01	0.02	-0.21	
	Perfectionism Org	0.01	0.01	.37	3.46	Perfectionism Org	0.02	0.01	.42*	4.90*	Perfectionism Org	0.02	0.01	.39	3.91
Step 3															
	Constant	0.26	1.11			Constant	0.29	1.01			Constant	0.26	1.01		
	Age	0.03	0.02	.35		Age	0.02	0.02	27		Age	0.02	0.02	.30	
	BMI	-0.01	0.04	-.03		BMI	-0.02	0.04	-.12		BMI	-0.02	0.04	-.15	
	PSS	-0.03	0.02	-.45		PSS	-0.01	0.02	-.19		PSS	-0.01	0.02	-.17	
	Perfectionism Org	0.03	0.01	.56		Perfectionism Org	0.02	0.01	.45		Perfectionism Org	0.02	0.01	.36	
	Perfectionism PS	-0.03	0.02	-.44		Perfectionism PS	-0.01	0.02	-.20		Perfectionism PS	-0.01	0.02	-.09	
	Perfectionism Total	0.01	0.01	.24	0.69	Perfectionism Total	0.01	0.01	.30	0.65	Perfectionism Total	0.01	0.01	.25	0.49

+ 35 minutes Step 1: $R^2 = .20$, $\Delta R^2 = .08$, $F(3,23) = 1.68$, $p = .20$; Step 2: $R^2 = .32$, $\Delta R^2 = .18$, $F(4,23) = 2.28$, $p = .08$, Step 3: $R^2 = 38$, $\Delta R^2 = .15$, $F(6,23) = 1.70$, p = .54
+ 50 minutes Step 1: $R^2 = .19$, $\Delta R^2 = .07$, $F(3,23) = 1.54$, $p = .24$; Step 2: $R^2 = .35$, $\Delta R^2 = .22$, $F(4,23) = 2.60$, $p = .07$, Step 3: $R^2 = .40$, $\Delta R^2 = .19$, $F(6,23) = 1.89$, p = .14
+ 60 minutes Step 1: $R^2 = .22$, $\Delta R^2 = .10$, $F(3,23) = 1.89$, $p = .16$; Step 2: $R^2 = .35$, $\Delta R^2 = .22$, $F(4,23) = 2.61$, $p = .07$, Step 3: $R^2 = .38$, $\Delta R^2 = .17$, $F(6,23) = 1.81$, p = .16
* $p < .01$
Perfectionism PS - Personal Standards, Perfectionism Org - Organisation

6.4.7.1.2 Stress Visit 2

Partial correlations (two-tailed) controlling for age and BMI revealed a significant negative relationship between Alexithymia EOT and salivary cortisol levels at 0, $r(18) = -.54$, $p = .02$, + 10, $r(18) = -.71$, $p = .001$, and + 25, $r(18) = -.45$, $p = .05$, minutes relative to visit commencement. No measures of perfectionism were revealed to have significant correlations with cortisol responsivity at Stress Visit 2.

Exploratory regression analysis revealed that the control variables (age, BMI, and PSS [Stress Visit 2]) significantly predicted baseline cortisol levels at 0 minutes relative to Stress Visit 2 commencement (step one). This first model accounted for 28% of the adjusted variance in cortisol levels at 0 minutes. Perceived Stress Scale (Cohen et al., 1983) score emerged as the only significant predictor in this first model. The addition of Alexithymia EOT at step two resulted in a significant model that accounted for an additional 46% of the adjusted variance (74% total variance). Alexithymia EOT and PSS score were the only significant predictors of cortisol levels at step two (see Table 6.6 for regression model values for cortisol measures).

At + 10 minutes the control variables (age, BMI, and PSS [Stress Visit 2]) no longer significantly predicted cortisol levels (step one). The addition of Alexithymia EOT at step two resulted in a significant model that accounted for 56% of the variance in cortisol levels at + 10 minutes relative to Stress Visit 2 commencement. Alexithymia EOT and PSS score were again the only significant predictors of cortisol levels at step two. No significant regression models were found for mid-stress salivary cortisol levels at +25 minutes.

Alexithymia EOT and PSS score were both negatively related to cortisol at 0 and + 10 minutes suggesting lower scores on these measures were associated with higher baseline pre-stress cortisol levels.

Considering that predictors of cortisol response have only emerged in relation to aggregated stress exposures in the previous studies (Kirschbaum, Bartussek et al., 1992; Pruessner, Gaab et al., 1997), the analyses were repeated with aggregated values. Salivary cortisol responses from both stress visits were aggregated by summation of AUCg, AUCi, and delta increase in line with the methods used by Pruessner, Gaab et al. (1997).

Partial correlations (two-tailed) controlling for age and BMI revealed a significant relationship between Perfectionism: Organisation and salivary cortisol AUCg, $r(18) = .45$, $p = .04$, and delta increase, $r(18) = .47$, $p = .03$. A significant relationship between

self-esteem and AUCg also emerged, $r(18) = .48$, $p = .03$. However, exploratory regression analysis (controlling for age, BMI, and summated PSS) produced no significant models for AUCg or delta increase (see Table 6.7).

Table 6.6 Hierarchical multiple regression analyses of relationships between Alexithymia EOT and salivary cortisol measures (controlling for age, BMI and PSS [Stress Visit 2])

	Measurement time point (relative to visit onset)											
	0				+ 10				+ 25			
	B	*SE B*	*β*	*F* change	*B*	*SE B*	*β*	*F* change	*B*	*SE B*	*β*	*F* change
Step 1												
	Constant	0.78	0.45		Constant	0.79	0.75		Constant	0.57	0.75	
	Age	0.01	0.01	.29	Age	0.01	0.02	.06	Age	0.01	0.02	.21
	BMI	-0.01	0.02	-.07	BMI	-0.004	0.03	-.03	BMI	0.00	0.03	-.03
	PSS	-0.02	0.01	-.64**	PSS	-0.01	0.01	-.28	PSS	-0.01	0.01	-.21
				3.54***				0.43				0.37
Step 2												
	Constant	0.86	0.27		Constant	0.93	0.48		Constant	0.66	0.66	
	Age	0.01	0.01	.25	Age	0.001	0.01	.01	Age	0.01	0.01	.18
	BMI	0.02	0.01	.17	BMI	0.03	0.02	.26	BMI	0.03	0.03	.18
	PSS	-0.03	0.010	-.76*	PSS	-0.02	0.01	-.41***	PSS	-0.01	0.01	-.31
	Alexithymia EOT	-0.03	0.01	-.68*	Alexithymia EOT	-0.04	0.01	-.82*	Alexithymia EOT	-0.03	0.01	-.52*
				30.40*				26.34*				5.63***

0 minutes Step 1: $R^2 = .38$, $\Delta R^2 = .28$, $F(3,20) = 3.54$, $p = .04$; Step 2: $R^2 = .78$, $\Delta R^2 = .74$, $F(4,20) = 14.84$, $p < .001$

+ 10 minutes Step 1: $R^2 = .07$, $\Delta R^2 = -.09$, $F(3,20) = 0.43$, $p = .73$; Step 2: $R^2 = .65$, $\Delta R^2 = .56$, $F(4,20) = 7.91$, $p = .001$

+ 25 minutes Step 1: $R^2 = .06$, $\Delta R^2 = -.10$, $F(3,20) = 0.37$, $p = .77$; Step 2: $R^2 = .29$, $\Delta R^2 = .13$, $F(4,20) = 1.76$, $p = .19$

*$p < .001$, **$p < .006$, ***$p < .04$

Alexithymia EOT - External Orientated Thinking

Table 6.7 Hierarchical multiple regression analyses of relationships between Perfectionism: Organisation, self-esteem and aggregated stress visit salivary cortisol measures (controlling for age, BMI and PSS [Stress Visit 1 + 2])

		AUCg					*Delta Increase*			
		B	*SE B*	*β*	*F* change		*B*	*SE B*	*β*	*F* change
Step 1										
	Constant	25.88	15.62			Constant	28.28	34.76		
	Age	0.53	0.37	.31		Age	0.90	0.83	.24	
	BMI	-0.35	0.63	-.14		BMI	-1.50	1.41	-.27	
	PSS	-0.22	0.17	-.31	1.68	PSS	-0.16	0.38	-.42	1.19
Step 2										
	Constant	16.35	17.47			Constant	34.27	32.49		
	Age	0.40	0.38	.23		Age	0.32	0.82	.09	
	BMI	-0.38	0.61	-.15		BMI	-1.89	1.33	-.34	
	PSS	-0.13	0.17	-.18		PSS	0.04	0.02	.02	
	Perfectionism: Org	0.23	0.18	.29		Perfectionism: Org	0.73	0.38	.43	3.79
	Self-esteem	0.34	0.27	.27	2.16					

AUCg Step 1: $R^2 = .22$, $\Delta R^2 = .09$, $F(3,21) = 1.69$, $p = .20$; Step 2: $R^2 = .39$, $\Delta R^2 = .19$, $F(5,21) = 2.01$, $p = .13$
Delta Increase Step 1: $R^2 = .17$, $\Delta R^2 = -.03$, $F(3,21) = 1.19$, $p = .34$; Step 2: $R^2 = .32$, $\Delta R^2 = .16$, $F(4,21) = 1.98$, $p = .14$

Whilst Perfectionism: Organisation did not significantly predict measures of cortisol response at Stress Visit 2, a median split of participants based upon Perfectionism: Organisation score revealed that high scorers demonstrated large elevations across both stress visits (see Figure 6.22). Thus, a loss of predictive capacity is likely due to the apparent sensitisation in response in participants scoring low in Perfectionism: Organisation at Stress Visit 2.

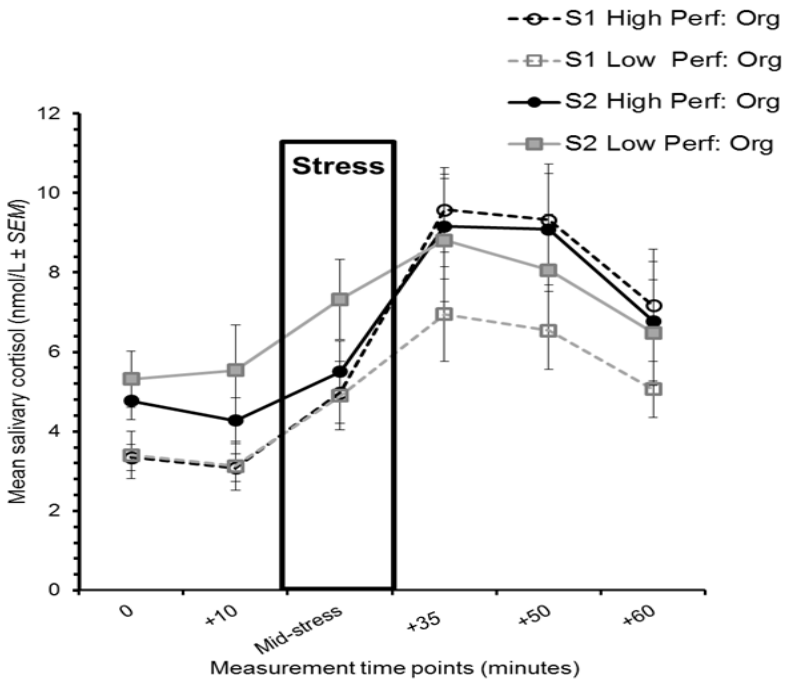


Figure 6.22 Mean (± *SEM)* salivary cortisol response profiles (nmol/L) across stress visits in high and low (median split) Perfectionism: Organisation participants

6.4.8 Capillary Blood Glucose Response

No significant differences were revealed between pre-and post-stress capillary blood glucose levels across study visits (controlling for age and BMI; visit, $F(2,45) = 0.21$, $p = .81$; time, $F(1,23) = 3.04$, $p = .10$) suggesting the standardised meals ensured a stable nutritional state across study visits (mean glucose values shown in 6.8).

Pearson's Product Moment (two-tailed) product-moment correlations revealed glucose levels were not significantly related to levels of salivary cortisol (measurement time points or aggregated measures).

Table 6.8 Mean capillary blood glucose (mmol/L) pre + post-stress across study visits

Study Visit	Capillary Blood Glucose (mmol/L)	
	+ 10 (pre-stress)	+ 35 (post-stress)
	Mean (SEM)	*Mean (SEM)*
Control	**6.54** (0.21)	**6.40** (0.24)
Stress visit 1	**6.13** (0.81)	**6.75** (0.24)
Stress visit 2	**6.16** (0.89)	**6.49** (0.19)

6.4.9 Cognitive Distraction/Interference and Cognitive Performance

A marginally significant trend for an effect of visit on ratings of general distracting thoughts whilst completing cognitive tests was revealed, $F(2,40) = 3.02$, $p = .06$. Post-hoc comparison revealed significantly heightened cognitive distraction whilst completing cognitive tests at Stress Visit 2 ($\overline{X} = 42.64 \pm 5.02$) compared to the non-stress control visit ($\overline{\overline{X}} = 27.17 \pm 4.81$; $p = .06$). Pearson's Product Moment's (two-tailed) product-moment correlations revealed cognitive distraction and interference VAS ratings were not significantly related to cognitive performance outcomes across any of the cognitive tests during either stress visit.

6.5 Discussion

6.5.1 Salivary Cortisol Response

Both stress visits elicited significant elevations in salivary cortisol above baseline levels (pre-stress) and levels elicited during a non-stress control day. The mean response across the whole sample suggests that the stress context was sufficient to elicit a comparable response during repeated exposures. A pre-stress anticipatory increase in response was evident at Stress Visit 2 which exceeded pre-stress levels at both the non-stress control visit and Stress Visit 1. Heightened anticipatory cortisol responses in men, but not women, in the anticipation of psychosocial stress (even if not actually exposed) have been previously reported (Kirschbaum, Wust, & Hellhammer, 1992). The heightened pre-stress response demonstrated in this all male sample is commensurate with this. Notably, this anticipatory response was not evident at Stress Visit 1. Prior to the first stress exposure, participants were not fully aware of what the 'challenging tasks' would entail. Whilst the exact nature of the stress tasks at the second stress visit was withheld, participants would have had more of an idea as to the likely protocol. This may underpin the heightened anticipatory response observed. Furthermore, an anticipatory response to stress exposure that comprises a physical challenge has been previously observed (Mason et al., 1973; Salvador, Suay, Gonzalez-Bono, & Serrano, 2003; Sutton & Casey, 1975).

Despite this heightened anticipatory response at Stress Visit 2, the post-stress responses (peak and recovery) were not significantly different across the stress visits in the whole sample. Inspection of the aggregated measures of cortisol (Stress Visits 1 and 2) further supports a comparable response across repeated exposures. Salivary cortisol AUCg, AUCi, and delta increase were significantly higher on both stress days compared to control. The heightened anticipatory response at Stress Visit 2 resulted in a higher overall total hormonal output (AUCg), and lower responsivity to stress exposure (AUCi and delta increase). However, the responses did not differ significantly.

Inspection of the individual salivary cortisol response profiles across stress visits suggests the lack of significant habituation observed in the whole sample is fairly representative of the individual responses elicited. Ten out of 21 (48%) participants demonstrated comparable responses across both stress visits (see Figure 6.6). Previous studies examining habituation responses across repeated laboratory stress exposures have reported a lack of habituation in 32% (Wust et al., 2005), 40% (Gerra et al., 2001),

and 35% (Kirschbaum et al., 1995) of samples. Only four participants (19%) demonstrated large reductions in cortisol response at Stress Visit 2 here. This compares to habituation in 65% (Kirschbaum et al., 1995), 60% (Gerra et al., 2001), and 52% (Wüst et al., 2005) of samples previously reported. A sensitisation of response in 19% of the sample is similar to that previously reported by Wust et al. (2005 [16%]). Three of the sample failed to demonstrate a cortisol response across both stress visits (14%).This translates to 68% of the sample demonstrating comparable or sensitised responses at a repeated stress exposure. It is difficult to disentangle the relative contribution of specific effects of manipulating the stress protocol in an attempt to reduce determinants of habituation, and variability in individual habituation characteristics of the sample. The findings may reflect the sample being comprised of a lower level of non-habituating cortisol responder 'types' than previous studies, rather than manipulated aspects of the stressor (e.g., contextual changes). However, the sample size recruited here was comparable to that reported by Kirschbaum et al. (1995) and Gerra et al. (2001; both *N* = 20).

An intervention period of six weeks was planned for the PL study (Study 4) due to evidence of slow accumulation of PL in the brain (Bruni et al., 1989). This informed the timescale between stress visits adopted here. The length of delay between repeated stressor visits has been previously shown to influence response habituation in animals and humans. Habituation in the majority of participants has been demonstrated after inter-stressor delays of 24 hr – 4 weeks (Engert et al., 2010; Gerra et al., 2001; Jonsson et al.; Kirschbaum, Pruessner et al., 1995; Schommer et al., 2003; von Kanel et al., 2006; Wust et al., 2005a). Petrowski et al. (2012) reported that an interval of 10 weeks was sufficient to reinstate previously habituated cortisol responses. It is worth noting that Petrowski et al. (2012) demonstrated no significant differences between cortisol responses during a second and third exposure to the TSST. However, the response magnitude provoked across these two exposures was significantly lower than that elicited by the initial exposure. Conversely, Hellhammer et al. (2012) reported TSST habituation after an inter-stress delay of 12 weeks. In the absence of data on response to the stress protocol administered here over varying inter-stressor intervals, it is not possible to disentangle the potential effects of stressor manipulations (e.g., addition of physical stressor) from the inter-stressor delay. Further studies would be needed to establish habituation patterns for this intervention with varying inter-stressor delays.

The contribution of the opposite sex panel to the observed cortisol response over repeated stress exposure cannot be fully determined from this study. The author is not

aware of any research which has examined the effect of panel sex over repeated exposures. Increased cortisol responsivity to a single opposite sex social-evaluative threat stressor has been previously demonstrated (Duchesne et al., 2012; Roney et al., 2010). Schwabe and Wolf (2010) have proposed that social evaluation may be likely to provoke the HPA axis under conditions in which individuals are concerned over self-presentation. This effect may be heightened when social evaluation comes from the opposite sex. Studies manipulating the sex of social-evaluative panels over repeated stress exposures would give further insight.

6.5.2 Cardiovascular responses

The laboratory stressor was sufficient to perturb cardiovascular parameters with significant elevations in SBP and DBP compared to baseline (pre-stress) and the non-stress control day, and HR compared to non-stress control. The BP response profiles were consistent in magnitude and response kinetics across both stress exposures. This was expected considering rapid response habituation may be specific to the HPA axis. Cardiovascular responses to acute stress provocation tend to be characterised by uniformity across repeated stress exposure (Mischler et al., 2005; Schommer et al., 2003). Additionally, this uniformity may persist over ten years (responses to cold pressor; Sherwood et al., 1997).

A review of the stability of cardiovascular responses suggests that correlations between repeated stress exposures are highest for HR (r = .60) and SBP (r = .51) responses; but lower for DBP response (r = .34; Manuck, 1994). However, the HR response elicited in this study was less consistent than for BP. Elevated HR levels during Stress Visit 1 did not reach significance (largely due to variability in HR) and significant variation in HR across Stress Visit 2 was reflective of heightened anticipatory HR levels. Heart rate was significantly raised immediately prior to, and immediately after, the speech task on both stress days compared to the control visit. A sudden drop to baseline and non-stress control levels was evident post-SECPT exposure. This reflects the passive coping nature of this task. The CPT requires passive tolerance and does not permit participants to exert control over the aversive stimuli. This type of stressor tends to instigate vasoconstriction resulting in increased BP and baroreceptor activation which combine to induce HR deceleration (Bosch et al., 2001). The increase in HR at + 50 and + 60 minutes may reflect the level of cognitive demand associated with the cognitive tests employed. Hence, cognitive tests requiring high mental effort often increase HR (Kennedy & Scholey, 2000).

6.5.3 Subjective Stress Response

The pattern of heightened anticipatory stress responses prior to the repeated stressor evident in the endocrine and physiological responses was further reflected in subjective responses. Significant increases in subjective stress and arousal (SACL) peaked post-stress induction at Stress Visit 1 suggesting that the stressor was psychologically threatening. The subjective stress response observed on Stress Visit 2 suggests that the stressor retained this capacity. Moreover, rather than habituating, subjective stress ratings pre- and post-stress were elevated. Subjective stress reached the peak magnitude of Stress Visit 1 response prior to stress induction and remained similarly elevated immediately post-stress. However, it is possible that a mid-stress peak was missed so significant habituation in peak response cannot be ruled out (Hellhammer & Schubert, 2012). Conversely, subjective arousal ratings were suggestive of habituated response by Stress Visit 2. This appears contradictory to evidence of significant and consistent sympathetic arousal demonstrated across both stress visits. However, as previously discussed (Section 4.5.4), and demonstrated across both Studies 1 and 2, correspondence between subjective appraisal and physiological stress response parameters is often weak (Campbell & Ehlert, 2012). Indeed, a lack of correlation between subjective and endocrine/physiological stress responses was again observed in this sample.

The lack of significant effects on subjective mood responses across stress visits is surprising. The short version of the POMS was previously employed in Study 2 and shown to be sensitive enough to index significant variations in mood states elicited by stress exposure. It may be hypothesised that exposure to a social-evaluative real-world stressor, is likely to be more closely linked to social identity and personal goals than a laboratory social-evaluative stressor, and therefore, a more potent agitator of subjective mood states. Greater subjective responses to a real-world public speech compared to a laboratory speech task have been previously reported (Dimsdale, 1984); albeit not employing the same measure of mood. However, the POMS has been previously shown to be sensitive to laboratory stress protocols (e.g., Serkan Het & Wolf, 2007; Markus et al., 1998).

6.5.4 Cognitive Performance

Stress exposure did not impair visual memory performance (VISGED). Whilst a pattern of lower performance following stress was evident, the sensitivity of this test (and visual

memory) to stress may be limited to older, high stress-load participants (Schubert at al., 2011) rather than potentially higher functioning young samples (Driscoll, Hamilton, Yeo, Brooks, & Sutherland, 2005).

No significant effects of stress exposure were revealed in the whole sample for 2-back performance. A non-significant trend for impaired performance compared to control was demonstrated for A-ospan performance on both stress days suggesting some sensitivity of this test to stress. Ceiling effects during the control, and to a lesser extent, during stress visits, may likely have limited variation in A-ospan performance across study visits. The expected task-switch test RT performance decrements across trial type were demonstrated across both control and stress visits. Reaction time for identification of targets was slower for switch trial vs. both repeat trial types under stress and non-stress conditions. Similarly, the decrement in RT switch cost performance between switch and repeat trials was significant across all visits and not further impaired by stress (replicating Plessow et al., 2012). The specific effects of stress on task-switch performance were demonstrated in performance accuracy decrements. Higher error rates on switch vs. pre-switch (repeat trials) were observed at Stress Visit 1, with a trend for a higher error rate (switch vs. nested) at Stress Visit 2. Furthermore, a significant accuracy switch cost performance decrement was evident between switch and repeat trials under conditions of stress. Such decrements are indicative of reduced efficiency of cognitive control processes under stress. This suggests stress impairs the cognitive control processes underpinning the flexible implementation of task goals required for accurate switching between tasks. Reduced accuracy in the absence of changes in RT across stress and control contexts suggests that the performance decrement was not indicative of a speed-accuracy trade-off (Duncko et al., 2009). Cognitive control may be a cognitive function that is inhibited when stress hormones interact to instigate the proposed switch in order to prioritise cognitive functions associated with coping with the stressor (e.g., consolidation). A comparable theoretical model of cognitive control impairment is proposed by Arnsten (2009) who considers stress to induce a shift from controlled top-down processing to automatic bottom-up processing under stress. This cognitive switch results in reduced availability of cognitive resources which impairs the flexible implementation of task goals.

Analysis of performance under conditions of stress in the highest cortisol responders revealed more consistent impairment in the tests of WM. A-ospan score and total were consistently impaired at both stress visits compared to control suggesting this test is specifically sensitive to impairment at high cortisol concentrations (and at low concentrations based on the results of Study 2). This is consistent with impaired ospan

WM performance and the positive correlation between impairment and cortisol levels reported by Schoofs et al. (2009). Similarly, impaired 2-back performance was evident when individual cortisol responsivity was examined. Target and total accuracy were impaired in the highest delta increase responders at Stress Visit 1. However, this effect was not maintained at Stress Visit 2. Schoofs et al. (2008) have previously reported the n-back may be sensitive to temporal effects under stress. The impairing effects of stress on performance diminished as a function of repetition (but not load; i.e., 2-back vs. 3-back). Hence the impairing effect of stress may have diminished as a function of repeated exposure by Stress Visit 2. However, the temporal effects reported by Schoofs et al., which occurred within a single test exposure, are unlikely to be as relevant over a temporal delay of six weeks.

Modest evidence of increased cognitive distraction during completion of cognitive tests during Stress Visit 2 was revealed. However, this heightened distraction was related to general distracting thoughts unrelated to stress exposure. Furthermore, no relationship between ratings of cognitive distraction/interference and cognitive performance were revealed (whole sample or high responders). This suggests the cognitive factors indexed by the distraction/interference measure employed here did not significantly contribute to the impaired cognitive function observed.

6.5.5 Predictors of Cortisol Responsivity

Perfectionism: Organisation emerged as the only significant predictor of aggregated measures of salivary cortisol response to the initial stress exposure. This adds to evidence from the preceding studies presented in this thesis, and the literature (Wirtz et al., 2007), such that dimensions of perfectionism may be associated with a tendency towards significant cortisol responses to acute stress. Consistent with previous evidence from the studies presented in this thesis, Perfectionism: Organisation emerged as the dimension of perfectionism associated with cortisol responsivity. This is inconsistent with the findings of Wirtz et al. (2007) who reported an association between cortisol response and a perfectionism dimension associated with excessive high standards and critical self-evaluation (Concern over Mistakes). Furthermore, consistent with the studies reported in Chapters 4 and 5, and inconsistent with Wirtz et al., no significant association between cortisol response and anticipatory cognitive appraisal measures was revealed. Neither the PASA nor PSRS were associated with subsequent cortisol response to stress. It is perhaps surprising that a heightened anticipatory response evident across endocrine, cardiovascular, and subjective parameters would be unrelated to anticipatory cognitive

appraisals; especially as transactional models consider cognitive appraisal processes to be key mediators of stress responses (Lazarus & Alfert, 1964; Lazarus & Folkman, 1984). This lack of relationship persisted over repeated stress exposures despite the likelihood that participants would be more able to accurately appraise threat and coping potential at Stress Visit 2. The smaller sample size compared to Wirtz et al., is acknowledged

The relationship between perfectionism and salivary cortisol response was not evident at Stress Visit 2. This appears to support the proposition that some of the heterogeneity in the evidence of an association between stable factors and cortisol responsivity may relate to initial stressor novelty (Epstein, 1986; Epstein & Obrien, 1985; Voigt, Ziegler, Grunertfuchs, Bickel, & Fehmwolfsdorf, 1990). Perfectionism: Organisation is associated with an increased need for order and control (Frost et al., 1990). This proclivity may be associated with higher cortisol responses in situations that are novel, and subsequently potentially lacking in order and controllability. Increased familiarity with the stressor context may thus abolish this association. However, in contrast to previous evidence (Bollini et al., 2004; Pruessner et al., 2005; Pruessner, Gaab et al., 1997), control orientation (LOC) has not emerged as a significant predictor of responses. Furthermore, whilst Perfectionism: Organisation was no longer a significant predictor of cortisol response at Stress Visit 2, participants scoring high on this dimension exhibited a comparable response across both stressors. A heightened pre- and post- stress response in participants with low Perfectionism: Organisation at Stress Visit 2 is likely to have reduced the predictive capacity of this dimension.

A negative association was revealed between the alexithymic EOT subscale score and perceived chronic stress (PSS), and Stress Visit 2 baseline salivary cortisol levels (0 and + 10 minutes). Despite some heterogeneity in the literature, the negative association between salivary cortisol responsivity and chronic stress has been previously reported. The relatively low chronic stress reported here is likely to have excluded individuals who may have demonstrated attenuated responses often associated with high levels of chronic stress/exhaustion (Kudielka, von Kanel, Preckel, Zgraggen, Mischler, & Fischer, 2006). Alexithymia DDF has been previously associated with significantly higher baseline salivary cortisol but not cortisol responsivity (de Timary et al., 2008). Despite elevated baseline levels, high DDF individuals exhibited analogous peak and recovery profiles to low DDF individuals. This suggests alexithymia subscales may be associated with anticipation of a stressor rather than the response itself. The association between EOT and baseline cortisol levels, but not mid/post-stress or aggregated response measures, revealed here appears to support such findings. Alexithymia EOT is characterised by an

emphasis on external, concrete stimuli rather than inner emotions and affective thinking (Franz et al., 2008). Hence high EOT individuals demonstrate a non-introspective cognitive style, a lack of imaginative thought, and a predilection towards concrete reality-based thinking (Bagby et al., 1994; Loas, Otmani, Verrier, Fremaux, & Marchand, 1996). High EOT individuals characterised by this cognitive style could conceivably be less likely to find the imagined aspect of the laboratory task stressful. Conversely, low EOT individuals may be characterised by high introspection, affective thinking, and more likely to engage with the imagined aspect of the laboratory stressor. It is noted that this specific alexithymic subscale has shown inconsistent internal validity when applied to samples reported within this thesis (r_{α} = .66 and .63; Studies 2 and 3 respectively) and in the literature (r_{α} = .55 – 66; Bagby et al., 1994; Bressi et al., 1996; Loas et al., 2001). The stability of the three-factor structure of the TAS-20 has also been questioned and a two-factor structure proposed (Erni, Lotscher, & Modestin, 1997; Loas, Otmani, Verrier, Fremaux, & Marchand, 1996). However, studies comprising larger samples that have assessed the reliability and factorial validity of the original three-factor structure model provide support for the reliability of this measure (Parker, Taylor, & Bagby, 2003).

Correlations between Perfectionism: Organisation and self-esteem, and cortisol responses (AUCg and delta increase) emerged when responses over both stress visits were aggregated. The emergence of self-esteem after aggregation of cortisol responses has been reported previously (Kirschbaum, Pruessner et al., 1995; Pruessner, Gaab et al., 1997). These trait factors did not significantly predict cortisol responsivity in subsequent regression analyses. Pruessner et al. (1997) reported increasing correlations between personality variables with increasing response aggregation over five TSST exposures when the first day was removed. Here aggregation comprised aggregated cortisol from only an initial and repeat stress exposure so it cannot be ruled out that further trait predictors would emerge over further exposures. However, as Pruessner et al. (1997) acknowledge, the use of aggregated data is not without controversy (Allen & Potkay, 1983; Day, Marshall, Hamilton, & Christy, 1983).

6.5.6 Standardisation of Nutritional State

The inclusion of a standardised study meal ensured participants had a comparable background nutritional state (indexed by capillary blood glucose) pre- and post-stress on both stress visits. However, it is acknowledged that more variability in glucose response may be likely in larger samples or in relation to BMI which was fairly consistent/varied little in the current study. No relationships were revealed between glucose level and

cortisol response, which is not surprising considering the lack of variability in glucose levels across the sample and visits. The inclusion of a standardised meal was considered a positive control measure to carry forward into the intervention study.

6.6 Interim Summary

A primary advantage of a laboratory stress protocol is the capacity to control variables known to modulate cortisol responsivity. This maximises the potential for the stressor to reliably provoke significant cortisol responses. The increased control offered by a laboratory setting likely increased the reliability in cortisol response elicited here. This study assessed the reliability of a stress context, manipulated in an attempt to reduce the contextual, physiological, and psychological factors associated with habituation to a repeated homotypic stressor, to provoke significant stress responses over repeated exposures. The findings suggest that this stress context would be suitable for the planned dietary intervention (Study 4). This study also provided further support for cognitive tests associated with the PFC being sensitive to stress exposure. Therefore, the n-back, A-ospan, and task-switch tests were selected as valid tests of cognitive function to administer in the subsequent dietary intervention study. Finally, further evidence of an association between perfectionism and cortisol responsivity added to the evidence accumulated in previous studies suggesting this personality trait may facilitate the selection of a sample likely to demonstrate heightened cortisol responsivity when confronted with a stressor.

Chapter 7:
Study 4

Chapter 7 Study 4 - A Randomised Placebo Controlled Trial Examining the Effects of Chronic (6 weeks) Phospholipid Intake on Cognitive Performance under Conditions of Acute Stress

7.1 Introduction

The final study presented in this thesis examined the potential for chronic (6 weeks) PL intake to moderate stress responses and cognitive performance under conditions of repeated acute laboratory stress exposure. This intervention study was informed by previous research undertaken in the thesis (Studies 1 – 3).

7.1.1 Identification of a Stress Context

Thesis Studies 1 and 2 highlighted the variability in the capacity of stress contexts to reliably provoke the HPA axis; confirming evidence from the literature (Biondi & Picardi, 1999; Dickerson & Kemeny, 2004). Whilst the potential benefits of examining stress responses and cognitive performance in real-world contexts is acknowledged, the lack of control and standardisation inherent in such studies increases the likelihood of inconsistent results. This would prove problematic for a dietary intervention as the small effects often observed maybe overshadowed by confounding variables associated with this potential inconsistency. The performing arts audition context did allow for collection of stress response markers and completion of cognitive tests during exposure to a fairly standardised stress context. However, this context was discounted owing to inconsistency in capacity to elicit a cortisol response. The benefits associated with greater control and standardisation inherent in laboratory stress protocols was evident in the more consistent results observed across repeated stress exposures in Study 3. A level of variability in cortisol response across repeated stress exposures is inevitable considering the inter- and intra-individual variability and tendency for rapid habituation in cortisol response (Kirschbaum et al., 1995; Petrowski et al., 2012; Pruessner et al., 1995; Schommer & Hellhammer, 2000; Wust et al., 2005b). However, responses to the laboratory stressor piloted in Study 3 suggest a level of consistency in response that was considered sufficient to warrant the application of this stress context in the dietary intervention study (both pre- and post-intervention).

7.1.2 Predictors of Cortisol Responsivity

Trait variables associated with cortisol responsivity were collected across the thesis to uncover stable predictors of cortisol response to acute stress provocation. This was undertaken with the intention of identifying stable characteristics associated with a tendency to exhibit heightened cortisol responsivity. This premise relies on the assumption that cortisol responsivity can be considered related to a constitutional disposition that underlies inter-individual differences in cortisol response to provocation (Schulz et al., 2005). This relationship is often assumed, owing to the transactional notion of stress emphasising an interaction between individual perception and appraisal (presumably mediated by dispositional factors) and stress demand. However, the evidence is often inconsistent (e.g., Blood et al., 1994; Kirschbaum, Bartussek et al., 1992; Salmon et al., 1989; Van Eck et al., 1996). An association between cortisol response and a sub-dimension of perfectionism emerged over the studies undertaken in this thesis. Perfectionism: Organisation was shown to be positively associated with cortisol responsivity both in a naturalistic and a laboratory social-evaluative context. No other stable characteristics were shown to be consistently associated with cortisol response. A dimension of alexithymia emerged as the only significant trait predictor of cortisol response to repeated stress exposure. However, in line with previous evidence (de Timary et al., 2008), this association was limited to early anticipatory rather than reactive cortisol responses.

The importance of assessing stable predictors of cortisol response over repeated stress exposures to account for the potential influence of situational variables such as initial stressor novelty has been emphasised (Harl et al., 2006; Misslin, Herzog, Koch, & Ropartz, 1982; Pfister, 1979; Simpkiss & Devine, 2003; Voigt et al., 1990). Indeed, perfectionism did not significantly predict cortisol responses to repeat laboratory stress suggesting that this dimension may be specifically related to stress responses to novel stimuli. However, the fact that individuals high in perfectionism showed little variation in response over repeated stress exposure suggests this measure may still be useful to predict a tendency towards high cortisol responses. Individuals scoring both high and low on this dimension of perfectionism demonstrated a general trend for marked cortisol increase during a repeated stress exposure. This may reflect the capacity of the stress context to induce sensitised responses over repeated exposures that may have diminished the predictive capacity of this trait dimension. The fact that Perfectionism: Organisation did not predict cortisol responses over repeated stress exposures suggests that whilst this characteristic is associated with an increased tendency towards cortisol

responsivity, it does not appear to be predictive of the habituation responder types previously identified (e.g., persistent high responses in non-habituators; Kirschbaum, Pruessner et al., 1995). This is confirmed in part by the observation that Perfectionism: Organisation only correlated with aggregated cortisol measures and did not contribute significantly to the regression models in Study 3 (see Section 6.4.7.1.2).

The consistent association between Perfectionism: Organisation and cortisol response (both net [AUCg] and reactivity [AUCi and delta increase]) in the studies presented in this thesis was considered sufficient to adopt this trait dimension of perfectionism to select participants for inclusion in the PL intervention study. Recent evidence has distinguished between anticipatory and reactive cortisol responder types and demonstrated an association between heightened anticipatory responses and greater cortisol responsivity (Engert et al., 2013). This would suggest that Alexithymia EOT may have a predictive capacity to identify heightened cortisol responsivity. However, the lack of predictive validity of alexithymia over previous studies resulted in the decision not to employ this trait factor to identify a sample for the intervention study. Moreover, the application of numerous selection criteria was considered too restrictive.

The rationale for attempting to identify predictors of increased cortisol responsivity relates to evidence that the effects of stress on cognitive performance are often only observed in individuals who exhibit high cortisol responses. The post-hoc identification of individuals demonstrating high cortisol responsivity often results in the impairing effects of stress on cognition being examined in a sub-set of a larger sample. Consequently, reported effects are often based on small groups (e.g., Buchanan et al., 2008, n = 6; Takahashi et al., 2006, n = 5). This was exemplified in WM memory performance observed in Study 3 which was significantly impaired only in the top 50th percentile of cortisol responders. Furthermore, evidence of the potential for PL intake to attenuate cortisol responses may operate as a function of cortisol response (i.e., high or low responsivity). Hellhammer et al. (2012) demonstrated a tendency for PL intake (administered with Omega-3 fatty acids) to attenuate cortisol responses in individuals with high cortisol responsivity and increase cortisol response in those with low responsivity. Thus PLs may have a normalising effect on cortisol response.

7.1.3 Identification of Cognitive Tests Sensitive to Stress

Evidence for the capacity for PL intake to protect cognitive performance under stress is fairly weak and inconsistent. This may partly be explained by divergent sensitivities of

tests of cognitive performance to stress. Therefore, a number of tests of cognitive performance were piloted across the thesis to identify candidate tests that demonstrate sensitivity to stress. In accordance with the literature (Elzinga and Roelofs, 2005; Schoofs et al., 2008; Luethi et al., 2008; Schoofs et al, 2009), tests of WM were demonstrated to be sensitive to stress. This impairment was observed both in the absence of cortisol (Study 2) and in those exhibiting the highest cortisol responses (Study 3). This may be indicative of cortisol operating in an inverted-U function as previously demonstrated in animal models (de Kloet et al., 1999) and human synthetic GC administration studies (Lupien et al., 1999; Young et al., 1999; see Section 1.4.3). Alternatively, impairment in the absence of cortisol may indicate that additional factors, such as cognitive interference associated with the psychological stress response, are underpinning impaired performance. This effect of cognitive interference reported in the literature (Stawski et al., 2006, 2009) was not replicated in this thesis. However, considering evidence of PL intake moderating the endocrine and psychological response to stress, PL supplementation has the potential to be protective of cognition in both cases.

The impairment of task-switching performance further supports the impairment of PFC-mediated cognitive function by stress. Reduced cognitive control, indexed by reduced performance accuracy when switching between tasks, appears sensitive to stress. Therefore, this executive function task and the tests of WM were considered suitably sensitive for use in this intervention study. The inclusion of both tests of WM was considered appropriate as both can be considered to measure distinct aspects of WM function. The n-back engages WM processes to actively maintain and dynamically rehearse target information. The A-ospan reflects WM capacity and measures the online processing of information whilst actively maintaining goal-relevant information.

7.2 Study Objectives and Hypotheses

This intervention study examined the potential for chronic (6 weeks) PL intake to moderate cognitive performance under conditions of laboratory stress. Perfectionist tendency was employed to select male participants with an increased likelihood of cortisol responsivity. Intake of a PL-rich drink was expected to proffer protective effects on cognitive performance tests sensitive to the impairing effects of stress compared to a matched placebo drink. This effect was expected to be moderated by attenuation of the cortisol response. Attenuation of the subjective psychological stress response was a secondary hypothesised effect of 6 weeks of PL intake.

7.3 Methods

7.3.1 Sample

Fifty-four healthy adult males were included in the study. Participants were recruited via the University of Leeds participant database and recruitment posters displayed on campus. After eligibility screening participants were randomly assigned to the PL-rich drink or placebo conditions. The characteristics of participants randomised across each drink condition are shown in Section 7.4.1.

7.3.2 Inclusion and Exclusion Criteria

All participants completed an online screening questionnaire prior to a secondary screening at the Institute of Psychological Sciences to ensure the study inclusion/exclusion criteria were met. Participants were recruited using the following criteria.

7.3.2.1 Inclusion criteria

- Male over 18 years of age
- Frost Multidimensional Perfectionism: Organisation score ≥ 13

7.3.2.2 Exclusion criteria

In addition to those stated in Methodologies Section 3.3.4:

- Medication use (prescribed and 'over-the-counter'). Intake of medication was recorded once participation had commenced. Participants detailed any medication intake started during study participation in their study (intervention drink) diary (Section 7.3.11). Participants found to have commenced the intake of psychotropic medications, steroids or any medication shown to affect HPA axis function during the drink intervention period were excluded. Other concomitant medication was permitted (e.g., paracetamol) but recorded and summarised for each drink condition in Section 7.4.1.
- Skin conditions (e.g., eczema) on hands and wrists (due to SECPT)
- Food allergies

- Participation in a clinical study within a month prior to screening or during participation

- Previous participation in a stress induction study

7.3.3 Design

The study conformed to a randomised, double-blind, placebo controlled, parallel groups design examining cognitive performance and acute physiological/subjective stress responses before and after a six week daily intake of a PL-rich or placebo drink. The study flow from screening to completion is shown in Figure 7.1.

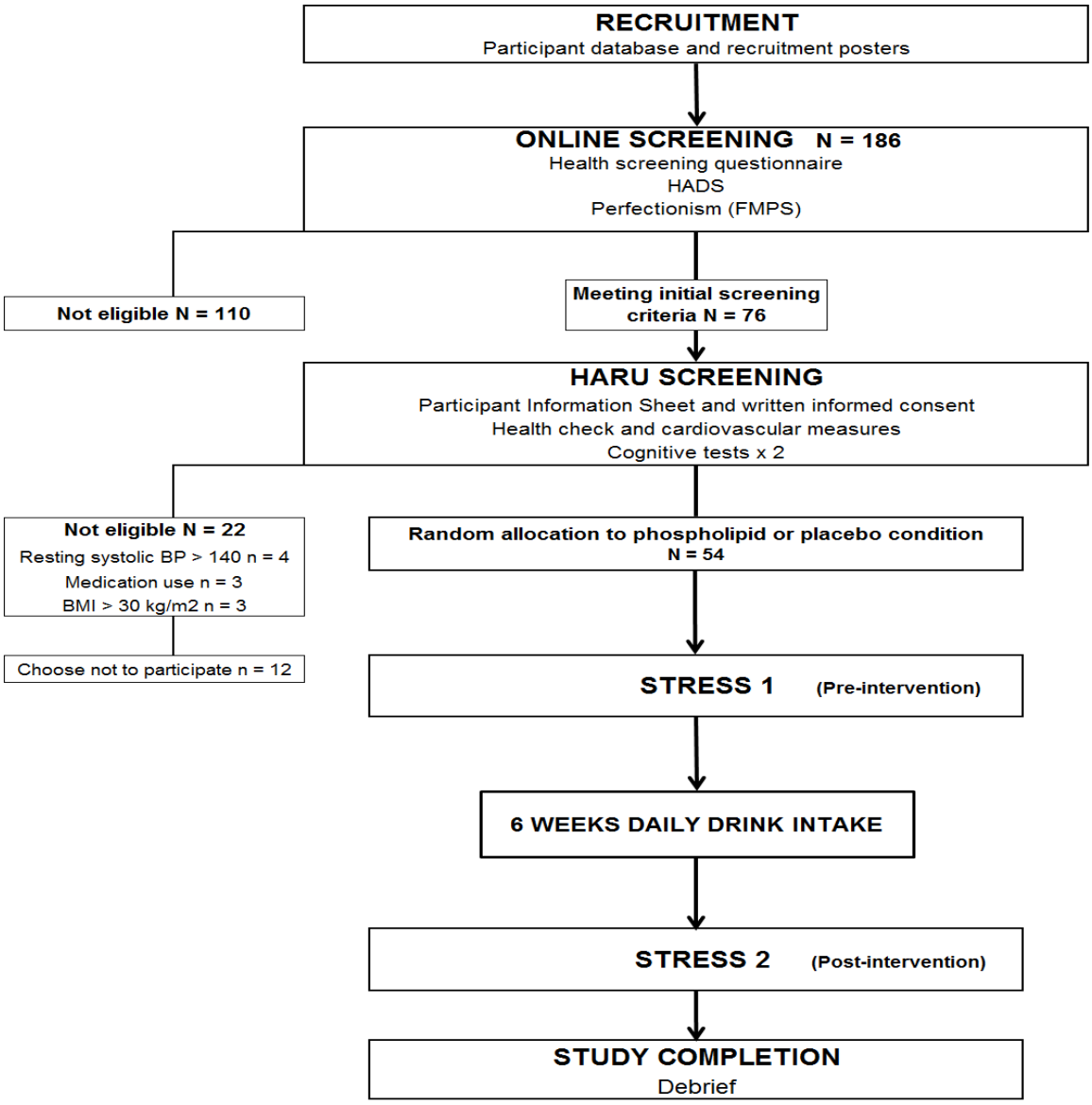


Figure 7.1 Intervention study flow diagram

7.3.3.1 Randomisation

A randomisation schedule was produced by the consulting statistician to ensure random allocation of participants to each drink condition. Participants were randomly assigned to the PL or placebo drink conditions after screening using this schedule. The statistician confirmed the statistical approach adopted but played no further role in the study. The randomisation schedule allowed for a dropout rate of 10%.

7.3.3.2 Condition blinding

Experimenters were blind to the drink conditions until all data were entered and checked and statistical analyses were completed. Intervention drinks were distinguished by a condition code (182 and 375) applied by the drink manufacturer at production. A sealed envelope containing the code of the intervention drinks was held by experimenters to allow a code break in the event of any major adverse reactions. Since there were no major adverse events this sealed envelope was returned sealed to the study sponsor upon completion of the study, together with a blinded report on the study outcome.

7.3.4 Endocrine Measures

7.3.4.1 Cortisol

Salivary cortisol was collected at 0, + 10, + 25 (mid-stress), + 35, + 45, and + 55 minutes relative to visit commencement across both stress visits. Salivary-free cortisol concentrations were determined using a Salivary Cortisol Enzyme Immunoassay kit (EIA; Sarstedt; Nümbrecht, Germany; described in Section 3.2.1.1). Intra- and inter-assay variability was below 6.60% and 9.97% respectively.

7.3.5 Physiological Measures

7.3.5.1 Blood pressure

A Spacelabs (model 90207) ambulatory blood pressure monitor was used to measure SBP, DBP, and HR at 0, + 10, + 15 (pre-speech), + 25 (mid-stress), + 30 (post SECPT), + 35, + 45, and + 55 minutes relative to visit commencement across both stress visits. Two measurements were taken at each time point and the average of the readings used in all analyses.

7.3.6 Subjective Measures

The following measures were employed to assess baseline chronic stress level and acute subjective responses to stress exposure. More detailed descriptions of measures are given in the General Methodologies section where indicated.

7.3.6.1 Perceived Stress Scale (PSS)

Participants completed the first PSS at the face to face screening ≤ 4 days prior to Stress Visit 1 (General Methodologies Section 3.3.1). A second PSS was completed at Stress Visit 2 to determine any changes in perceived chronic stress levels following drink intake.

7.3.6.2 The Trier Inventory for Chronic Stress – Long English (TICS-LE)

The TICS-LE (Schulz, Schlotz, & Becker, 2004) is a 57 item self-report measure that assesses nine specific interrelated factors of chronic psychosocial stress: *Work Overload, Social Overload, Pressure to Perform, Work Discontent, Excessive Demands at Work, Lack of Social Recognition, Social Tensions, Social Isolation, and Chronic Worrying.* A *Chronic Stress Screening Scale* can be calculated by summing items from the Work Overload, Social Overload, Excessive Demands at Work, Chronic Worrying, Lack of Social Recognition subscales. Item responses are made with reference to a five-point Likert scale: *never* = 0*, rarely* = 1*, sometimes* = 2, *often* = 3, *very often* = 4. Participants are required to indicate how often they have faced a number of stress-related situations or experiences within the last three months (e.g., I receive too little appreciation for my accomplishments). Internal consistency ranging from $r\alpha$ = .84 to .91 has been reported (Schulz et al., 2004).

The PSS was adopted as the primary measure of perceived chronic stress across all studies reported in this thesis. This measure was primarily selected as it has been widely employed and validated (Almadi, Cathers, Mansour, & Chow, 2012; Andreou et al., 2011; Cohen et al., 1983; Luft, Sanches, Mazo, & Andrade, 2007). The TICS is a validated scale (Petrowski, Paul, Albani, & Braehler, 2012; Schlotz et al., 2011) that has been increasingly adopted as a measure of chronic stress. Considering this measure has been employed to identify high chronic stress-load participants in previous PL intervention studies (Hellhammer et al., 2010; Schubert et al., 2011), the TICS was additionally employed at screening to compare chronic stress scores across the respective scales. The Chronic Stress Screening Scale score was calculated for this comparison. It was anticipated that the TICS scores would provide additional confirmation that individuals

experiencing very high levels of chronic stress (excluded on the basis of a score ≥ 30 on the PSS across studies in this thesis) were not recruited into the study.

7.3.6.3 Stress and Arousal Checklist (SACL)

The SACL was completed at 0, + 10, + 25, + 35, + 45, and + 55 minutes relative to visit commencement across both stress visits. A mid-stress measure was added in acknowledgment of evidence of peak subjective stress occurring mid-stressor, and greater correlations between ratings at this time point and physiological responses (Hellhammer & Schubert, 2011; General Methodologies Section 3.2.3.1).

7.3.6.4 Profile of Mood States (POMS)

The POMS was completed at 0, + 10, + 35, and + 45 minutes relative to visit commencement across both stress visits (General Methodologies Section 3.2.3.2).

7.3.6.5 Cognitive distraction/interference VAS

Four cognitive distraction/interference VAS were employed to explore the potential influence of cognitive factors on cognitive performance (previously described in Section 6.3.6.5).

7.3.7 Trait Personality Measures

7.3.7.1 Frost Multidimensional Perfectionism Scale (FMPS)

The Perfectionism: Organisation subscale of the FMPS (Frost et al., 1990) was employed to select participants who may be more likely to demonstrate high cortisol responsivity to acute stress. A median split of Perfectionism: Organisation scores collected over previous studies undertaken in this thesis (N = 57) was used to identify the Organisation score for the top 50^{th} percentile of participants. Accordingly, only individuals scoring ≥ 13 on the Perfectionism: Organisation subscale were considered eligible for participation in this intervention study. Acceptable reliability coefficients were found for subscales: Parental Expectation (rα = .69), Organisation (rα = .86), Concern over Mistakes (rα = .86), Personal Standards (rα = .77), and Total Perfectionism Score (rα = .87). The subscales Parental Criticism (rα = .65) and Doubts about Actions (rα = .67) were found to be less reliable. However, these subscales were not employed in the selection of individuals eligible for participation.

7.3.8 Standardised Test Meal

The same standardised meal administered during Study 3 (previously described in Chapter 0) was given to participants one hour prior to each stress visit to standardise nutritional status. All meals were well tolerated and fully consumed.

7.3.9 Tests of Cognitive Performance

All cognitive tests described below were administered twice during screening and once upon completion of the stress protocol. A full description of each test is detailed in the General Methodologies Chapter 3.

7.3.9.1 2-back

As only trends in impairment were demonstrated following stress exposure in Study 3 (whole sample), an adjustment was made to this test in an attempt to increase sensitivity to the impairing effect of stress. The inter-stimulus delay was reduced from 1000 ms (Study 2 and 3) to 850 ms.

7.3.9.2 A-ospan

Due to evidence of ceiling effects demonstrated during Study 3 (see Section 6.3.9.2), 5 set size operations were added to increase task difficulty. The version employed here comprised five stimulus blocks of 3, 4 and 5 set size operations (15 operations in total). The maximum score for A-ospan score and total was 60.

7.3.9.3 Task-switch test

The task-switch test was administered as per Study 3 (see Section 3.5.3.4 for description of this test).

7.3.10 Intervention drinks

Participants were randomly allocated to a bovine milk-derived PL-rich concentrate or placebo drink at study entry. Both intervention drinks were provided by Arla Foods. The PL-rich and placebo were both water-based drinks produced with milk protein concentrated powder. Drinks were flavoured with vanilla and nougat and contained 1.5% added sucrose to give a comparable taste. The macronutrient content of both products was similar, providing approximately 140 kcal per daily portion (250 ml). See Table 7.1 for macronutrient composition across drinks.

Table 7.1 Macronutrient composition per 100g test product

Nutrient	Placebo	Phospholipid-rich drink
Protein	3.1g	3.3g
Carbohydrate	6.2g	6.0g
Fat	2.0g	1.7g

The PL-rich drink was formulated using a milk protein concentrate rich in phospholipids (Arla Foods Ingredients) which provided a daily dose of 2.7 g of PLs (including 300 mg PS). The placebo drink did not contain any PLs. The fat content of the placebo drink was matched with the PL-rich drink by adding butteroil, which contains only triglycerides. The PL composition of the PL-rich drink was comparable to the PL composition found naturally in bovine milk (see Table 7.2 for relative PL compositions). The PL-rich drink contained a concentration of PLs over 100 times that found naturally in normal whole bovine milk.

Test products were provided in plain white 250ml TetraBrik® cartons each with a separate straw. Both drinks were labelled with the date of manufacture and a 3-digit blinding code.

Table 7.2 Relative % PL composition of PL drink (PL-rich) and PL pool in whole bovine milk

Phospholipid profile	Composition of phospholipid (% of total PL)	
	PL-rich	Bovine milk*
Phosphatidylcholine	27	28.7
Phosphatidylethanolamine	22	31.4
Phosphatidylserine	12	11.2
Sphingomyelin	27	19.9
Phosphatidylinositole	8	3.6
Other	4	4.5

* Taken from Garcia et al., 2012

Participants were requested to consume the drink every day in the morning for six weeks. Consumption later in the day was permitted if participants forgot to consume the drinks in the morning. The drinks were suitable for adding to cold foods (e.g., cereals) but due to instability when heated, participants were told not to add the drinks to hot beverages.

7.3.11 Intervention Drink Diary

Participants were asked to complete a daily self-report study diary during consumption of the test drinks (Appendix 22). The diary recorded the consumption of drinks each day (self-reported adherence), time of consumption, intake of any medication, and adverse events across the six weeks intervention period (adverse events shown in results Section 7.4.1). The drink diary was used to monitor intake of any medication associated with altered HPA axis function during the drink intervention period.

Each participant was provided with a test diary and initially 17 test drinks in a cool bag. Seventeen drinks were provided to allow sufficient stock to last two weeks. Extra drinks were issued in case there was any delay in participants completing their restock/compliance visits. Participants returned to the Institute of Psychological Sciences twice over the six week drink intake period to restock drinks and collect new diaries. A member of the research team met participants face to face at each restock visit to discuss any issues arising and to check that the drinks were being tolerated and consumed as required.

7.3.12 Procedure

Eligibility was initially assessed by completion of an online screening questionnaire. The online questionnaire provided participants with an overview of what the study entailed. Information on respondents' health and medication intake status was collected at this point. The FMPS was also completed as part of the online questionnaire to permit selection of individuals with potential for increased stress responsivity (FMPS score ≥ 13). Individuals meeting the study inclusion criteria were invited to the Institute of Psychological Sciences to complete a secondary screening visit to confirm eligibility. Participants were familiarised with all study measures at this visit and provided written informed consent after prior reading of the participant information sheet. Four BP/HR measures were collected to exclude participants exhibiting raised BP (> 140/90 mmHg over repeated measures). The PSS was also administered to exclude individuals experiencing high chronic stress (≥ 30). The 2-back, A-ospan and task-switch test were completed twice during the screening visit. Weight and height were measured to calculate BMI.

The stress visit procedural timeline for specific measurement time points across stress visits is shown in Figure 7.2. All study visits commenced between 1100 hr and 1600 hr

(collection of cortisol measures and cognitive testing occurred between 1200 hr and 1720 hr). A standardised test meal and water was consumed upon arrival at the Institute of Psychological Sciences. Participants relaxed in a testing cubicle for one hour after consumption of the meal. After completion of the relaxation period an ambulatory BP monitor was fitted to the upper non-dominant arm of each participant. Salivary cortisol, cardiovascular and subjective response measures (SACL and POMS) were collected at timed intervals across each visit. Participants were exposed to the stress induction protocol previously detailed in section 6.3.10.1. Following completion of the stress induction period the cognitive tests were completed in serial order. Cortisol, cardiovascular and subjective measures were collected in between each cognitive test. The cognitive interference/suppression VAS were administered after all cognitive tests were completed (+ 65 minutes).

A partial debrief was given to participants following completion of Stress Visit 1 explaining that none of the 'recorded' data would be analysed until completion of Stress Visit 2. Provision of study drinks and diary are described in Section 7.3.11. Participants returned six weeks (± 2 days) after Stress Visit 1 to complete Stress Visit 2. The start time of the stress visits was matched within 1 hour to control for any time of day effects. A full debrief was provided upon completion of Stress Visit 2.

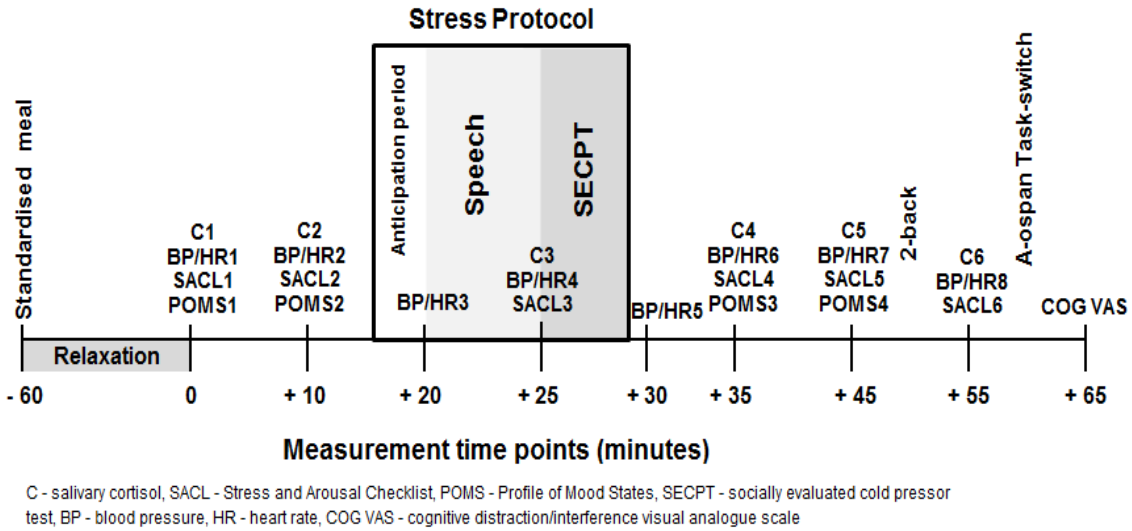


Figure 7.2 Stress visit procedural timeline

7.3.13 Ethical Approval

All participants provided written informed consent prior to study inclusion. An initial outline of the study was given to potential participants in an email disseminated on the university-wide participant database and to those replying to study recruitment posters. Interested individuals were directed to an online screening questionnaire to assess eligibility for participation. All online data were treated with strict confidentiality and stored on secure encrypted computers.

Ethical considerations related to the stress protocol have been previously outlined (Section 6.3.11). In an attempt to encourage compliance with intervention drink consumption, participants were falsely told that saliva samples might be taken to check that the drinks were being consumed. Participants were informed that no such tests took place at the full debrief following completion of Stress Visit 2.

The study was approved by the University of Leeds' Institute of Psychological Sciences Research Ethics Committee (Ref: 12-0163; submitted and approved October 2012). An honorarium of £120 was paid upon completion of the study.

7.3.14 Statistical Analyses

Cortisol data were skewed and normalized using logarithmic transformations. One participant from the PL condition was removed from the study due to non-compliance with study drink intake. All data from this participant were removed from analysis. The final sample comprised 27 participants in the placebo condition (182) and 26 in the PL-drink condition (375). Participants demonstrating performance on cognitive tests significantly below the sample mean (> 3.29 SD) were removed from the analyses of that specific test. Removal of cognitive test performance outliers is detailed in relevant test results sections below. Independent T-tests were employed to compare participant characteristics at baseline according to drink condition and between conditions post-intervention.

The SAS mixed models procedure (PROC MIXED) was employed to analyse the effects of stress exposure on salivary cortisol, cardiovascular (SBP, DBP and HR), and subjective stress parameters (POMS, SACL), and across trial for the task-switch test. Participant ID was entered as a random effect; drink condition, visit, time, and trial (task-switch test only), were fixed effects. Age, BMI, and PSS scores (pre- and post-intervention) were initially entered as covariates but subsequently removed from models due to non-significance. Tukey-Kramer-adjusted *p* values (Tukey, 1951) were employed

to compare least-squares mean responses across and between the profiles of each drink condition.

The SAS mixed models procedure (PROC MIXED) was also employed to compare salivary cortisol, cardiovascular, and subjective stress parameters, and cognitive performance outcomes between drink conditions post-intervention at Stress Visit 2. The corresponding measure of each dependent variable at Stress Visit 1 was entered as a covariate in each model to control for pre-intervention levels of each measure. Participant ID was entered as a random effect; drink condition, and time were fixed effects. Tukey-Kramer-adjusted *p* values (Tukey, 1951) were employed to compare least-squares mean post-intervention stress responses and performance outcome measures between drink conditions.

The method of selecting covariance structure for the mixed models is described in General Methodologies Section 3.6.2.

Pearson's Product Moment (two-tailed) correlations were employed to explore the relationship between subjective stress ratings (SACL) and cortisol and cardiovascular responses.

7.4 Results

7.4.1 Participant Characteristics

Independent T-tests revealed no significant differences between participants randomised to the PL and placebo drink conditions in terms of age, HADS-A, PSS (pre-intervention), and Perfectionism: Organisation, at pre-intervention baseline (see Table 7.3 for participant characteristics according to drink condition and *p* values). A significant difference in HADS-D score across condition was revealed, $t(51) = 2.22$, $p = .03$. Participants in the PL condition reported significantly higher depression ratings compared to those in the placebo condition. However, the HADS-D scores for both conditions were both well within the 'non-caseness' range (< 7; Bjelland et al., 2002) and likely inconsequential.

No significant pre-intervention differences were revealed across drink condition for cold pressor hand submersion time during the SECPT at Stress Visit 1.

Table 7.3 Participant characteristics across drink condition at baseline (N = 54)

Participant Characteristic		Drink Group Condition		
		PL-drink	Placebo	
		X (SEM)	*X (SEM)*	*p* value
Age		**22.04** (0.76)	**20.81** (0.34)	.14
BMI		**22.60** (0.39)	**23.18** (0.38)	.30
HADS- A		**4.26** (0.39)	**4.15** (0.48)	.61
HADS- D		**2.80** (0.41)	**1.52** (0.28)	.03
PSS	Pre-intervention	**14.80** (1.00)	**13.45** (0.70)	.18
TICS (CSSS)	Pre-intervention	**14.19** (1.05)	**11.90** (1.11)	.14
Perf: Org		**16.31** (0.81)	**16.63** (0.80)	.78
Cold pressor time	Stress visit 1	**135.48** (11.13)	**138.78** (11.05)	.75

PSS - Perceived Stress Scale
HADS - Hospital Anxiety Depression Scale.
TICS - Trier Inventory for Chronic Stress
Perf: Org - Perfectionism: Organisation

A Pearson's Product Moment (two-tailed) correlation revealed a positive correlation between the PSS and TICS (CSSS), $r(53) = .51$, $p < .001$. Comparable scores between the two measures of chronic stress were observed across both drink conditions at study entry (see Table 7.3). No significant differences between participants in the PL and placebo drink conditions for TICS (CSSS) score were found. Scores above 15 (Hellhammer, Hero, Franz, Contreras, & Schubert, 2012) and 16 (Schult, Hero, & Hellhammer, 2010) on the TICS have been previously reported to represent elevated chronic stress-load (Hellhammer et al., 2012). Mean CSSS scores in both drink condition groups were below these cut-off points.

7.4.1.1 Post-intervention characteristics across drink condition

No significant differences were revealed between duration of hand submersion across drink condition at Stress Visit 2, $t(51) = 0.04$, $p = .97$. No significant differences were revealed across drink condition for number of test drinks consumed, $t(51) = -1.08$, $p = .29$. Self-reported adherence to test drink consumption was very good (PL-drink [*mean ± SEM]*, $X = 41.12 \pm 0.43$; placebo, $X = 41.76 \pm 0.32$). Drinks were well-tolerated and no serious adverse events related to consumption were reported. No intake of medication associated with moderation of HPA axis function was reported during study drink consumption. An overview of reported medication intake and adverse events is shown in Figure 7.3.

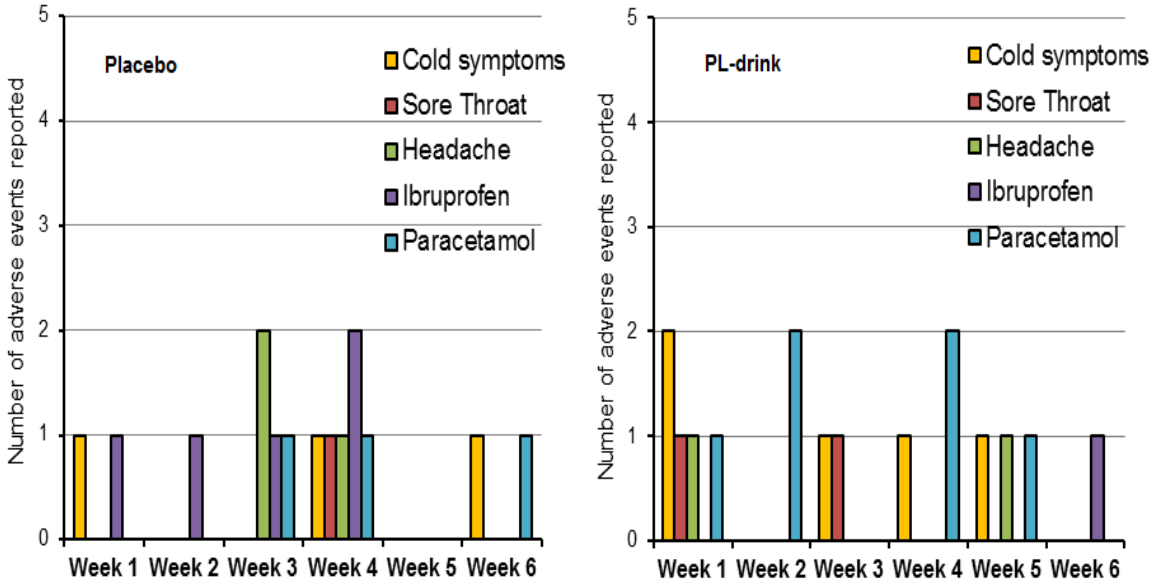


Figure 7.3 Frequency of reported adverse events (illness and medication intake) across drink intervention period

7.4.2 Salivary Cortisol Response

7.4.2.1 Effects of stress exposure on salivary cortisol

A significant condition×visit×time interaction, $F(16,248) = 2.25$, $p = .01$, was demonstrated for the profiles of salivary cortisol response across the two stress visits. This significant interaction was reflected in main effects of time, $F(5,260) = 57.38$, $p < .001$, and visit, $F(1,52) = 9.18$, $p = .01$. However, no significant effect of drink condition was found, $F(1,51) = 2.44$, $p = .12$ (see Figure 7.4).

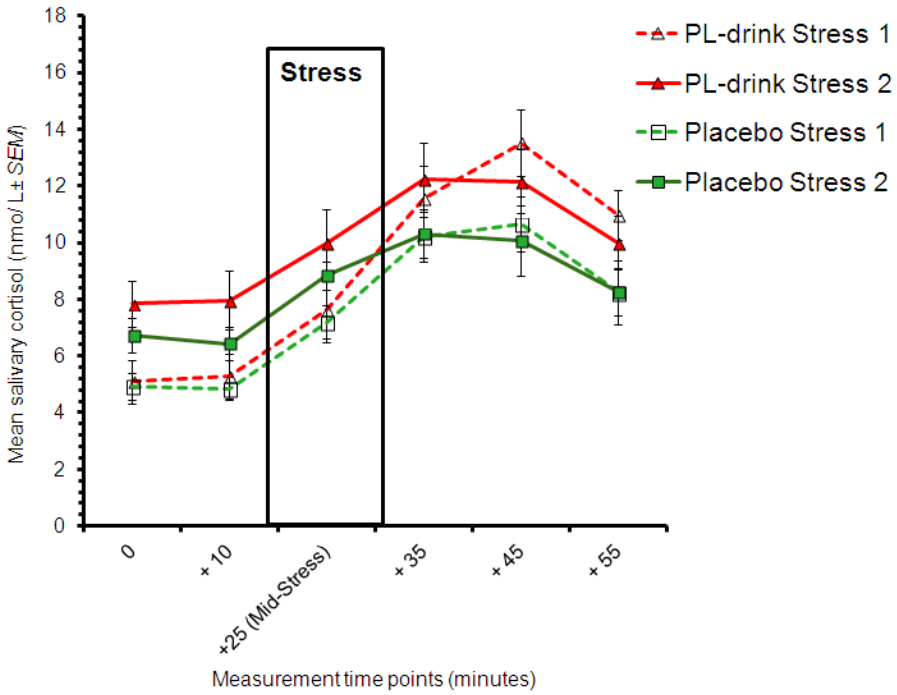


Figure 7.4 Mean (± *SEM)* salivary cortisol response (nmol/L) according to drink condition and stress visit

A pattern of a higher post-stress response trajectory and peak (+ 35, + 45, and + 55 minutes) cortisol was demonstrated during Stress Visit 1 in the PL-drink condition. However, differences between salivary cortisol levels across the response profile at Stress Visit 1 were not significant. The significant condition×visit×time interaction reflects a post-intervention increase in anticipatory salivary cortisol at Stress Visit 2. Whilst this tendency was demonstrated in both drink conditions, this response sensitisation only reached significance in the PL-drink condition. Salivary cortisol levels at 0 minutes were significantly higher than corresponding levels at Stress Visit 1 for this drink condition (significant at $p = .04$).

Within the salivary cortisol profile at Stress Visit 1, both drink conditions demonstrated significantly higher cortisol levels at + 25, + 35, + 45, and + 55 minutes compared to pre-stress levels at 0 and + 10 minutes (all significant at $p < .05$). In the PL-drink condition cortisol levels continued to rise significantly post-stress, with levels at + 35 and + 45 minutes significantly higher than mid-stress (+ 25 minutes) levels. The less pronounced post-stress rise in cortisol in the placebo condition resulted in no further significant increases from mid-stress (+ 25 minutes) onwards.

The heightened anticipatory salivary cortisol levels at Stress Visit 2 resulted in a less pronounced increase to peak than that demonstrated at Stress Visit 1. This was reflected in only cortisol levels at + 35 and + 45 minutes being significantly higher than pre-stress levels at 0 and + 10 minutes in both drink conditions (all significant at $p < .05$).

7.4.2.2 Aggregated measures of cortisol response

No significant differences in salivary cortisol AUCg across stress visits and between drink conditions were demonstrated (Figure 7.5). Higher anticipatory pre-stress cortisol levels and subsequent less pronounced rise to peak at Stress Visit 2 resulted in smaller AUCi and delta increase at Stress Visit 2 for both drink conditions. This difference was significant for the PL-drink condition reflected by a main significant effect of visit for AUCi, $F(1,51) = 8.07$, $p = .01$, and delta increase, $F(1,51) = 9.35$, $p = .003$, (Figure 7.6). Post-hoc comparisons revealed significantly lower AUCi and delta increases in the PL-drink condition at Stress Visit 2 compared to Stress Visit 1 ($p < .03$). A comparable response pattern in the placebo condition did not reach significance.

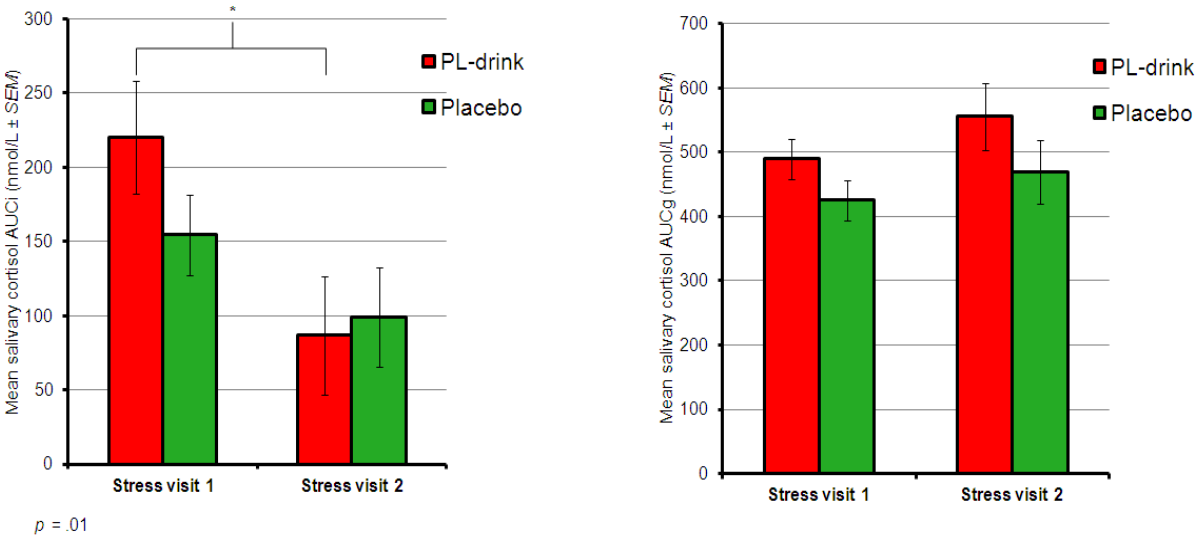


Figure 7.5 Mean (± *SEM)* salivary cortisol AUCi and AUCg according to drink condition and stress visit

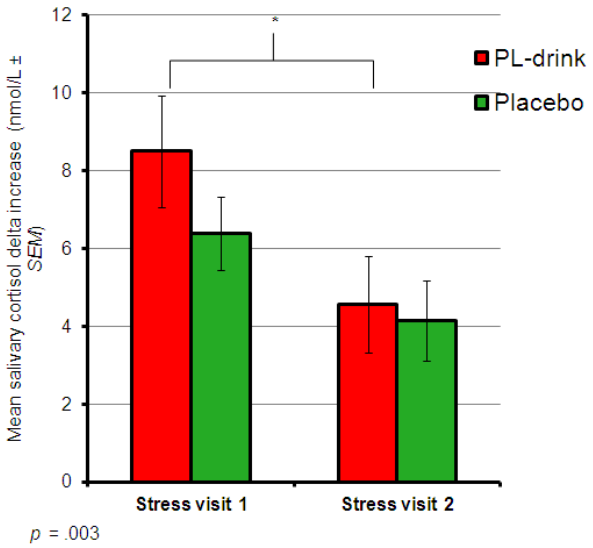


Figure 7.6 Mean (± *SEM)* salivary cortisol delta increase according to drink condition and stress visit

7.4.2.3 Effect of drink condition on salivary cortisol response

Mixed models ANCOVAs controlling for salivary cortisol response at Stress Visit 1 (baseline) revealed baseline cortisol was a significant predictor of cortisol levels from + 10 minutes onwards, and cortisol AUCg, at Stress Visit 2 (*F* statistics shown in Table 7.5). No significant effect of drink condition was found for salivary cortisol levels across the post-intervention visit response profile or aggregated measures.

Table 7.4 *F* statistics for relationship between salivary cortisol at Stress Visit 1 baseline (covariate) and cortisol at Stress Visit 2 (post drink-intervention)

Measurement time point (minutes)	*F* value*	*p*
+ 10	8.24	< .001
+ 25	35.65	< .001
+ 35	40.32	< .001
+ 45	34.71	< .001
+ 55	26.15	< .001
AUCg	34.49	< .001

*df = 1,49

7.4.3 Cardiovascular Response

7.4.3.1 Effects of stress exposure on SBP

A significant main effect of time, $F(7,364) = 143.84$, $p < .001$, visit, $F(1,52) = 11.16$, $p < .001$, and a trend for condition×time×visit interaction, $F(22,348) = 1.49$, $p = .07$, were revealed for the profiles of SBP response across the two stress visits. No significant effect of drink condition was found, $F(1,51) = 1.63$, $p = .20$ (see Figure 7.7).

No significant differences were revealed across the SBP response profile between drink conditions at Stress Visit 1 suggesting a comparable response kinetic across the drink conditions at baseline. Higher baseline pre-stress SBP was evident in the PL-drink condition at Stress Visit 2. This anticipatory SBP response at + 10 minutes was significantly higher than the corresponding SBP measures at Stress Visit 1 ($p = .01$). However, from + 20 minutes onwards a comparable overall response profile was demonstrated across both stress visits. Significantly higher SBP at + 20, + 25, and + 30, compared to pre-stress levels at 0 and + 10 minutes during Stress Visit 2 were found (all significant at $p < .001$). Systolic BP was still sufficiently raised by + 35 to be significantly higher than pre-stress SBP at + 10 minutes ($p < .001$). However, SBP had dropped significantly lower than peak SBP levels by this point. Systolic BP at + 35, + 45, and + 55 minutes was significantly lower than SBP at + 20, + 25, and + 30 minutes (all significant at $p < .03$).

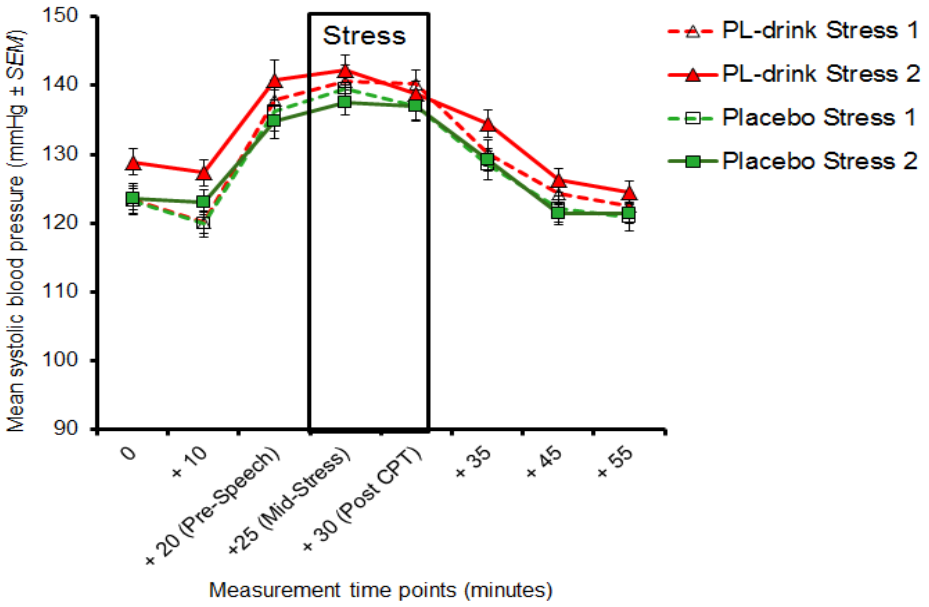


Figure 7.7 Mean (± *SEM)* SBP (mmHg) according to drink condition and stress visit

No heightened anticipatory response was evident at Stress Visit 2 for the placebo condition. Post-hoc comparisons of the SBP profile for both stress visits revealed significantly higher SBP at + 20, + 25, and + 30, compared to pre-stress levels at 0 and + 10 minutes (all significant at $p < .001$). Systolic BP at + 35 minutes was sufficiently raised to be significantly higher than SBP at + 10 minutes ($p < .001$). However, SBP at + 35, + 45, and + 55 had dropped significantly below peak SBP levels at + 20, + 25, and + 30 minutes (all significant at $p < .001$).

7.4.3.2 Effect of drink condition on SBP

Mixed model ANCOVAs controlling for SBP at Stress Visit 1 (baseline) revealed baseline SBP was a significant predictor of SBP across all measurement time points at Stress Visit 2 (*F* statistics shown in Table 7.5).

Table 7.5 *F* statistics for relationship between SBP at Stress Visit 1 baseline (covariate) and SBP at Stress Visit 2 (post drink-intervention) at each measurement time point

Measurement time point (minutes)	*F* value*	*p*
0	20.21	< .001
+ 10	15.21	< .001
+ 20	25.83	< .001
+ 25	43.74	< .001
+ 30	41.59	< .001
+ 35	47.57	< .001
+ 45	34.47	< .001
+ 55	27.05	< .001

*df = 1,49

A significant main effect of drink condition was revealed for SBP responses at 0, $F(1,50) = 4.27$, $p = .04$, and + 35 minutes, $F(1,50) = 4.72$, $p = .03$. Participants consuming the PL-drink had significantly higher SBP at both time points during Stress Visit 2 (both significant at $p < .04$). The post-intervention (Stress Visit 2) increase in SBP demonstrated in PL-drink condition at these time points was not evident in the placebo condition (See Figure 7.8).

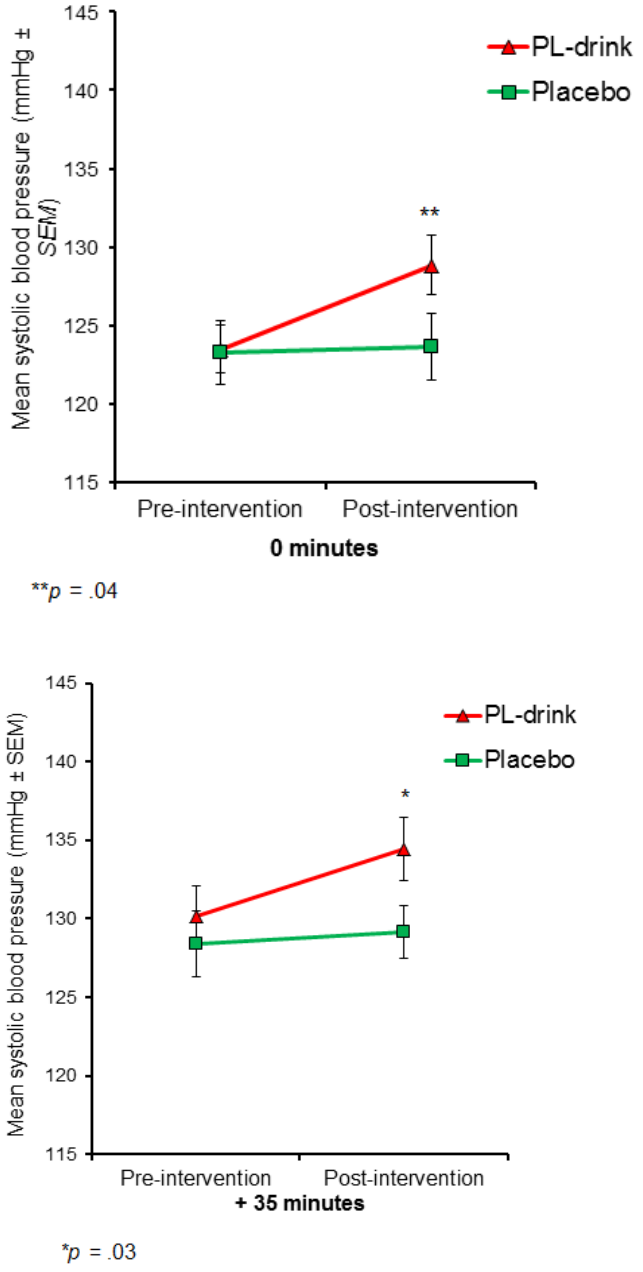


Figure 7.8 Mean SBP (± *SEM)* pre + post intervention according to drink condition at 0 and + 35 minutes

A marginally significant trend for a main effect of drink condition was also revealed for SBP responses at + 45, $F(1,50) = 3.68$, $p = .06$, minutes. Participants consuming the PL-drink had higher SBP at this time point during Stress Visit 2 compared to the placebo condition (significant at $p < .06$; see Figure 7.9).

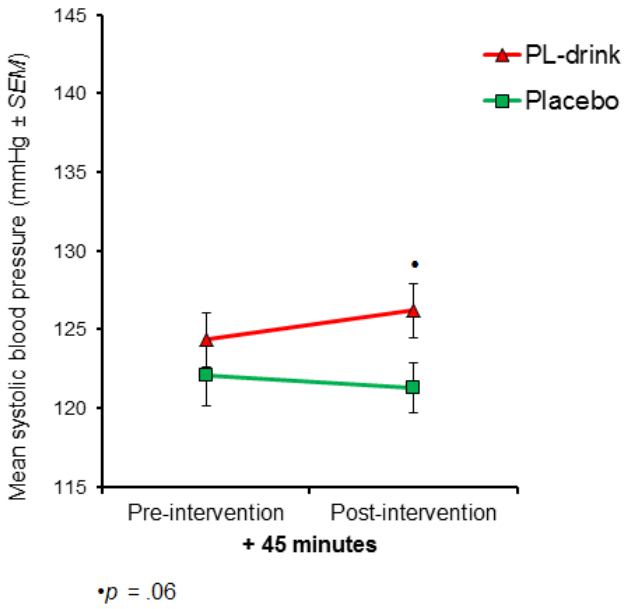


Figure 7.9 Mean SBP (± *SEM)* pre + post drink intervention according to drink condition at + 45 minutes

7.4.3.3 Effects of stress exposure on DBP

A significant main effect of time, $F(7,364) = 93.07$, $p < .001$, and visit, $F(1,52) = 9.86$, $p < .002$, was revealed for the profiles of DBP response across the two stress visits. The effect of drink condition, $F(1,51) = 2.23$, $p = .12$, and condition×time×visit interaction, $F(22,348) = 1.05$, $p = .42$, were non-significant (see Figure 7.7).

No significant differences were found across the DBP response profile between drink conditions at Stress Visit 1 suggesting a comparable response kinetic across the drink conditions pre-intervention.

Post-hoc comparisons revealed an analogous Stress Visit 1 DBP response profile across both drink conditions. Significantly higher DBP at + 20, + 25, + 30, and + 35, compared to pre-stress levels at 0 and + 10 minutes during Stress Visit 1 were demonstrated in both drink conditions (all significant at $p < .04$). Diastolic BP also declined sufficiently by + 35, + 45, and + 55 minutes to be significantly lower than peak DBP at + 20, +25, and + 30 minutes (all significant at $p < .01$).

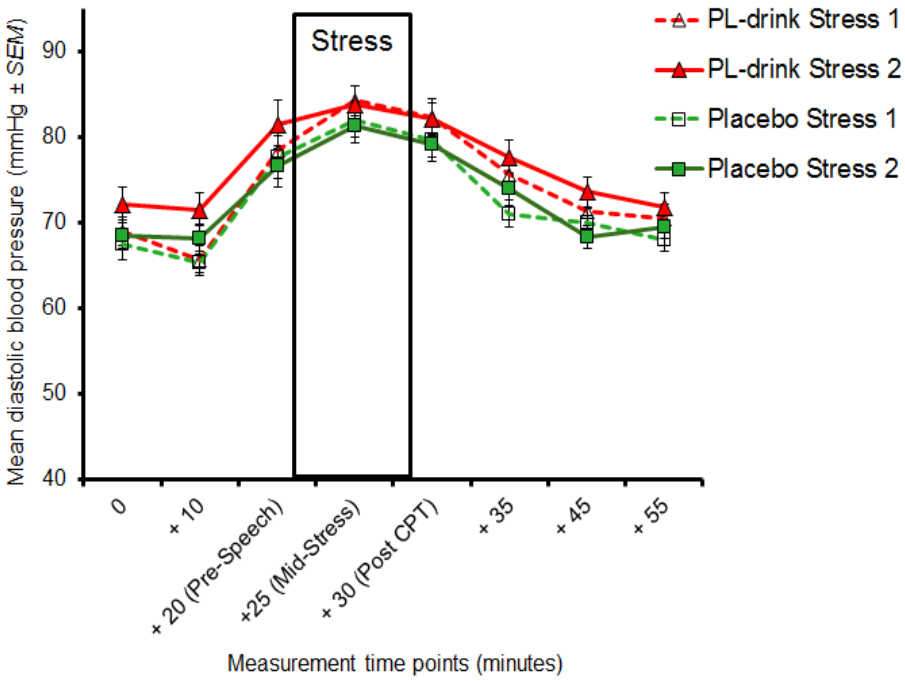


Figure 7.10 Mean (± *SEM)* DBP (mmHg) according to drink condition and stress visit

A trend towards higher pre-stress baseline DBP (0 and + 10 minutes) was evident for the PL-drink condition during Stress Visit 2. Diastolic BP was significantly higher at + 20, + 25, and + 30, compared to pre-stress levels at 0 and + 10 (all significant at $p < .001$). A slower post-stress decline in DBP than that demonstrated during Stress Visit 1 was evident. Diastolic BP had recovered to levels significantly lower than peak DBP (+ 20, +25, and + 30 minutes) by + 45 and + 55 minutes (whereas this response recovered by + 35 minutes at Stress Visit 1).

Post-hoc comparisons of the DBP profile for Stress Visit 2 in the placebo condition also revealed significantly higher SBP at + 20, + 25, and + 30, compared to pre-stress levels at 0 and + 10 minutes (all significant at $p < .001$). Additionally, DBP was significantly lower at + 35 minutes than peak response at + 25 ($p = .001$). Diastolic BP had dropped significantly below peak levels at + 20, +25, and + 30 minutes by + 45 and + 55 minutes (all significant at $p < .001$).

7.4.3.4 Effect of drink condition on DBP

Mixed model ANCOVAs controlling for DBP at Stress Visit 1 (baseline) revealed baseline DBP to be a significant predictor of DBP across all measurement time points at Stress Visit 2 (*F* statistics are shown in Table 7.6).

Table 7.6 *F* statistics for relationship between DBP at Stress Visit 1 baseline (covariate) and DBP at Stress Visit 2 (post-intervention) at each measurement time point

Measurement time point (minutes)	*F* value*	*p*
0	5.63	< .02
+ 10	10.17	< .002
+ 20	17.48	< .001
+ 25	43.74	< .001
+ 30	45.11	< .001
+ 35	36.25	< .001
+ 45	18.28	< .001
+ 55	10.69	< .002

*df = 1,49

A significant main effect of drink condition, $F(1,50) = 3.83$, $p = .02$, and a marginally significant baseline×condition interaction, $F(1,50) = 3.83$, $p = .06$, were revealed for DBP at + 45 minutes. Homogeneous DBP levels were demonstrated + 45 minutes at Stress Visit 1. However, participants consuming the PL-drink had significantly higher DBP at this time point during Stress Visit 2 (significant at $p = .01$). Participants consuming the PL-drink demonstrated an increase in DBP post-intervention, whilst a decrease was evident in the placebo condition (see Figure 7.11).

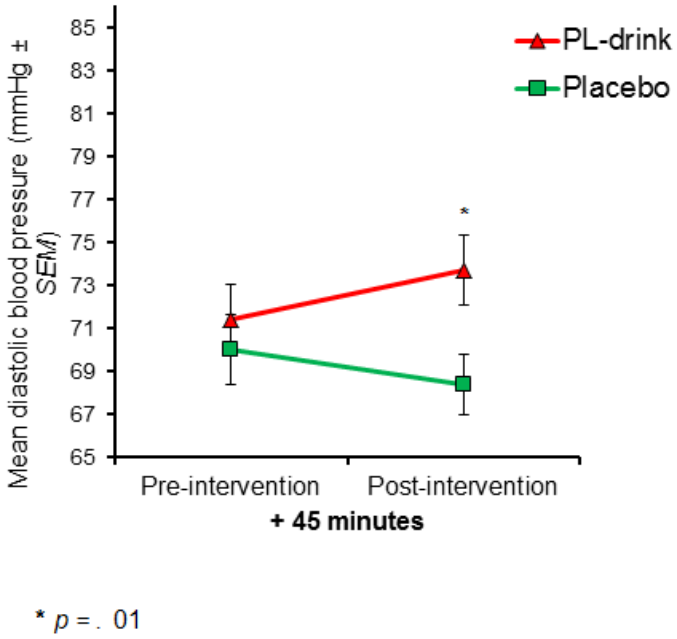


Figure 7.11 Mean (± *SEM)* DBP pre + post drink intake according to drink condition at + 45 minutes

7.4.3.5 Effects of stress on HR

A significant main effect of time, $F(7,364) = 30.84$, $p < .001$, and visit, $F(1,52) = 33.60$, $p < .001$, were revealed for the profiles of HR response across the two stress visits. A non-significant effect of drink condition, $F(1,51) = 0.01$, $p = .95$, and a non-significant condition×time×visit interaction, $F(22,348) = 0.53$, $p = .96$, were observed (see Figure 7.12).

Heart rate peaked during the speech anticipation period across all stress visits in both drink conditions. No significant differences were found between drink conditions at Stress Visit 1 for HR response profile suggesting a comparable response kinetic across the drink conditions at baseline.

Post-hoc comparisons confirmed an analogous HR response profile at Stress Visit 1 across both drink conditions. Heart rate at + 20 was significantly higher than that at 0 minutes pre-stress, and + 30, + 35, + 45, and + 55 minutes post-stress in both drink conditions (all significant at $p < .04$).

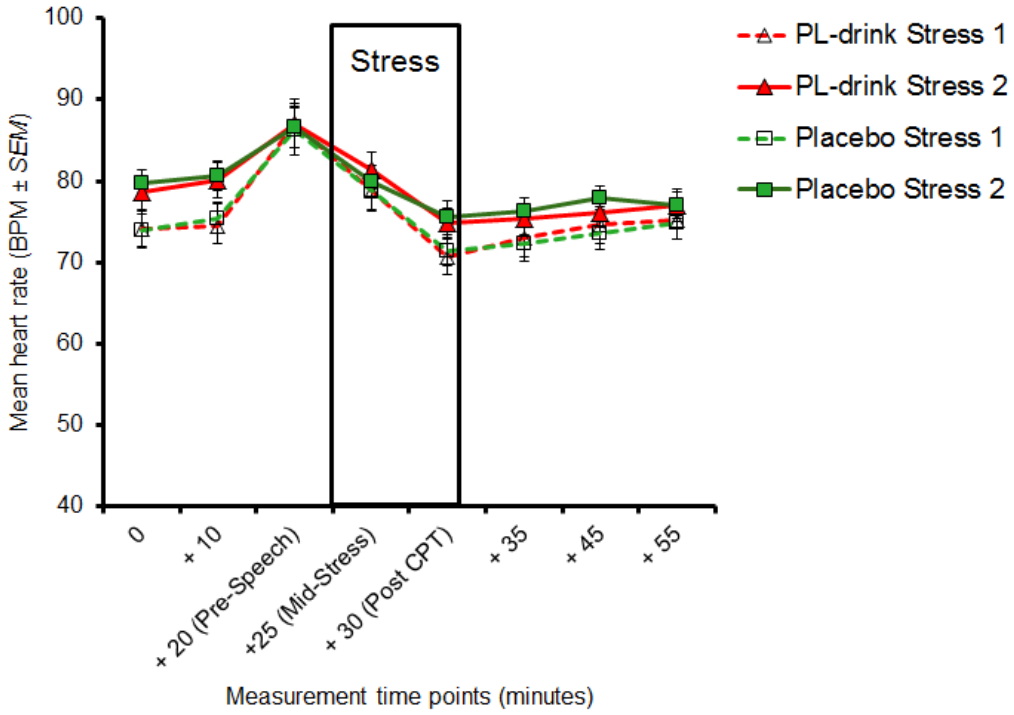


Figure 7.12 Mean (± *SEM)* HR (bpm) according to drink condition and stress visit

Figure 7.12 shows the higher baseline HR (0 and + 10 minutes) during Stress Visit 2 in both drink conditions but these differences were non-significant ($p > .72$). Heart rate in the PL conditions was significantly higher at + 20 compared to 0 minutes pre-stress, and + 30, + 35, + 45, and + 55 minutes post-stress (all significant at $p < .001$). The elevated baseline HR levels in the placebo condition resulted in no significant differences between pre-stress levels at 0 and + 10 minutes and peak at + 20 minutes. The HR post-stress response profile was comparable to that demonstrated across all other stress visits. Heart rate was significantly lower at + 30, + 35, + 45, and + 55 minutes compared to response peak at + 20 minutes (all significant at $p < .001$).

7.4.3.6 Effect of drink condition on HR

Mixed model ANCOVAs controlling for HR at Stress Visit 1 (baseline) revealed baseline HR was a significant predictor of HR across all measurement time points during Stress Visit 2 (*F* statistics are shown in Table 7.7). No effect of drink condition was revealed across the HR response profile.

Table 7.7 *F* statistics for relationship between HR at Stress Visit 1 (covariate) and HR at Stress Visit 2 (post-intervention) at each measurement time point

Measurement time point (minutes)	*F* value*	*p*
0	10.44	< .001
+ 10	16.51	< .001
+ 20	7.54	< .001
+ 25	9.77	< .001
+ 30	6.10	< .001
+ 35	20.24	< .001
+ 45	21.36	< .001
+ 55	10.58	< .002

*df = 1,49

7.4.4 Subjective Responses

7.4.4.1 Subjective stress response to stress (SACL)

A significant condition×time×visit interaction, $F(16,248) = 10.78$, $p < .001$, was revealed for the profiles of subjective stress response across the two stress visits (Figure 7.13). The significant interaction reflected a less pronounced subjective stress response, characterised by higher baseline pre-stress ratings and a dampened mid-stress peak, demonstrated across both drink conditions at Stress Visit 2. For PL-drink supplemented participants, subjective stress ratings at + 10 minutes were significantly higher, and subjective mid-stress ratings at + 25 minutes significantly lower, than the corresponding ratings during Stress Visit 1 (both significant at $p < .01$). A more consistent pre-stress anticipatory subjective stress response was demonstrated by participants in the placebo condition. Stress ratings at both 0 and + 10 minutes were significantly higher, and subjective mid-stress ratings at + 25 minutes were significantly lower, than corresponding ratings during Stress Visit 1 ($p < .001$).

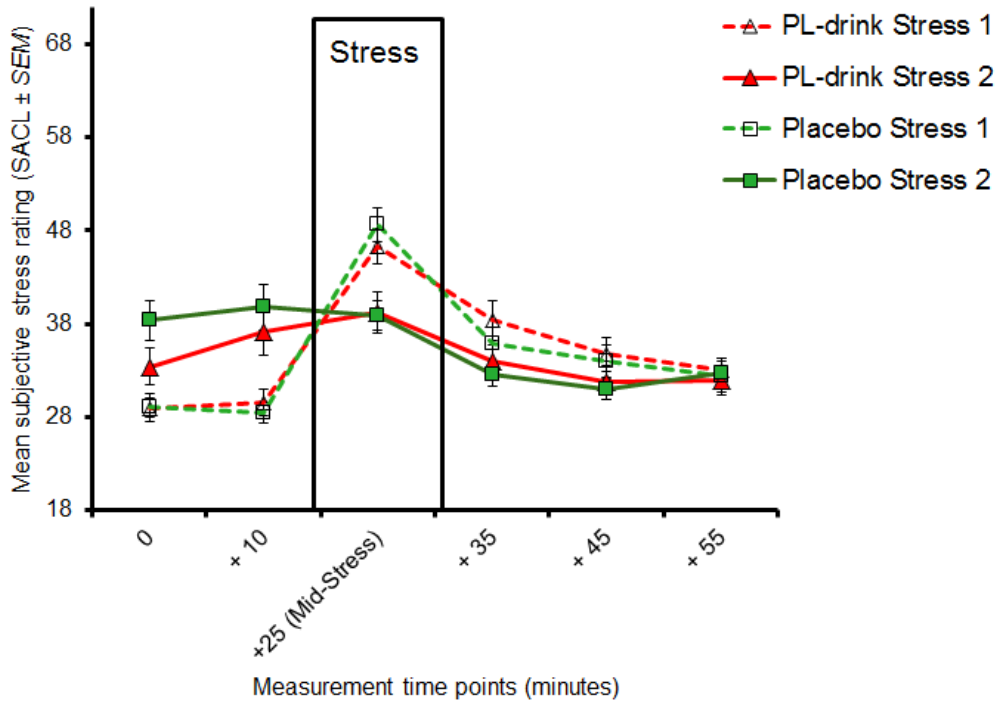


Figure 7.13 Mean (± *SEM)* subjective stress rating (SACL) according to drink condition and stress visit

There was also a significant main effect of time, $F(5,260) = 47.03$, $p < .001$. Post-hoc comparisons revealed stress ratings at + 25, and + 35 minutes were significantly higher than pre-stress ratings at 0 and + 10 minutes in both conditions during Stress Visit 1 (all significant at p <.001). The peak in subjective stress rating at + 25 minutes was also significantly higher than post-stress ratings at + 35, + 45, and + 55 minutes (all significant at $p < .001$).

During Stress Visit 2 subjective stress peaked mid-stress at + 25 minutes in the PL-drink condition, which was significantly higher than post-stress ratings at + 45 and + 55 minutes (both significant at $p < .02$). Conversely, a pre-stress peak was demonstrated in the placebo condition. This resulted in stress ratings at 0 and + 10 minutes being significantly higher than post-stress levels at + 35 and + 45 minutes (all significant at p < .01). Stress ratings at the pre-stress + 10 minutes peak were also significantly higher than + 55 minutes ($p = .01$). Mid-stress subjective stress at + 25 minutes was significantly higher than post-stress ratings at + 35 and + 45 (both significant at $p < .01$).

The effects of drink condition, $F(1,51) = 0.03$, $p = .90$, and visit, $F(1,52) = 0.10$, $p = .75$, were non-significant.

7.4.4.2 Effect of drink condition on subjective stress (SACL)

Mixed model ANCOVAs controlling for subjective stress rating at Stress Visit 1 (baseline) revealed baseline subjective stress was a significant predictor of subjective stress across all measurement time points at Stress Visit 2 (*F* statistics are shown in Table 7.8).

Table 7.8 *F* statistics for relationship between subjective stress rating (SACL) dimension ratings at Stress Visit 1 baseline (covariate) and ratings at Stress Visit 2 (post-intervention) at each measurement time point

Measurement time point (minutes)	Stress (SACL)	
	F value*	*p*
0	18.09	< .001
+ 10	19.73	< .001
+ 25	18.46	< .001
+ 35	38.66	< .001
+ 45	26.72	< .001
+ 55	19.51	< .002

*df = 1,49

A significant main effect of drink condition, $F(1,50) = 4.50$, $p = .05$, and a significant baseline×condition interaction, $F(1,50) = 6.12$, $p = .02$, were revealed for subjective stress ratings at 0 minutes. Participants consuming the PL-drink demonstrated a marginally significant trend for lower subjective stress ratings at 0 minutes during Stress Visit 2 than those consuming the placebo ($p = .06$; see Figure 7.14).

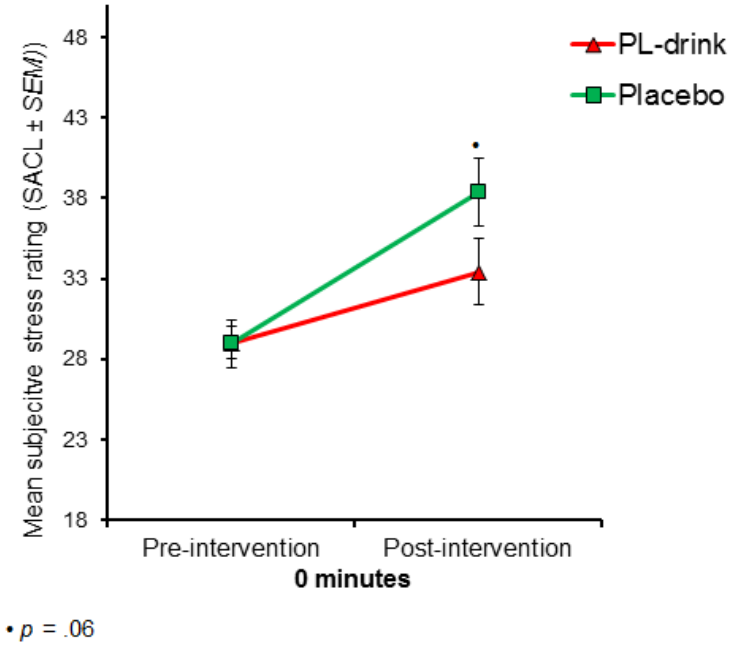


Figure 7.14 Mean (± *SEM)* subjective stress (SACL) pre + post intervention according to drink condition at 0 minutes

7.4.4.3 Subjective arousal response to stress (SACL)

A significant main effect of time, $F(5,260) = 18.73$, $p < .001$, and a trend for an effect of visit, $F(1,52) = 3.38$, $p = .07$, were revealed for the profiles of subjective arousal across the two stress visits. No significant effect of drink condition, $F(1,51) = 0.63$, $p = .43$, and a non-significant condition×time×visit interaction, $F(16,248) = 1.16$, $p = .30$, were observed (Figure 7.15).

No significant differences were revealed across the subjective arousal response profile between drink conditions at Stress Visit 1 suggesting a comparable response kinetic across the drink conditions at baseline.

Post-hoc comparisons revealed no significant differences across the response profile during Stress Visit 1 in the PL-drink condition. However, in the placebo condition peak arousal ratings at + 25 minutes were significantly higher than pre-stress ratings a 0, and + 10 minutes, and post-stress ratings at + 35, + 45, and + 55 minutes (all significant at $p < .03$).

This relationship was reversed at Stress Visit 2. Peak arousal ratings at + 25 minutes were significantly higher than pre-stress ratings at 0 and + 10 minutes in the PL-drink

condition (both significant at $p < .03$). No significant differences were found across the placebo condition response profile.

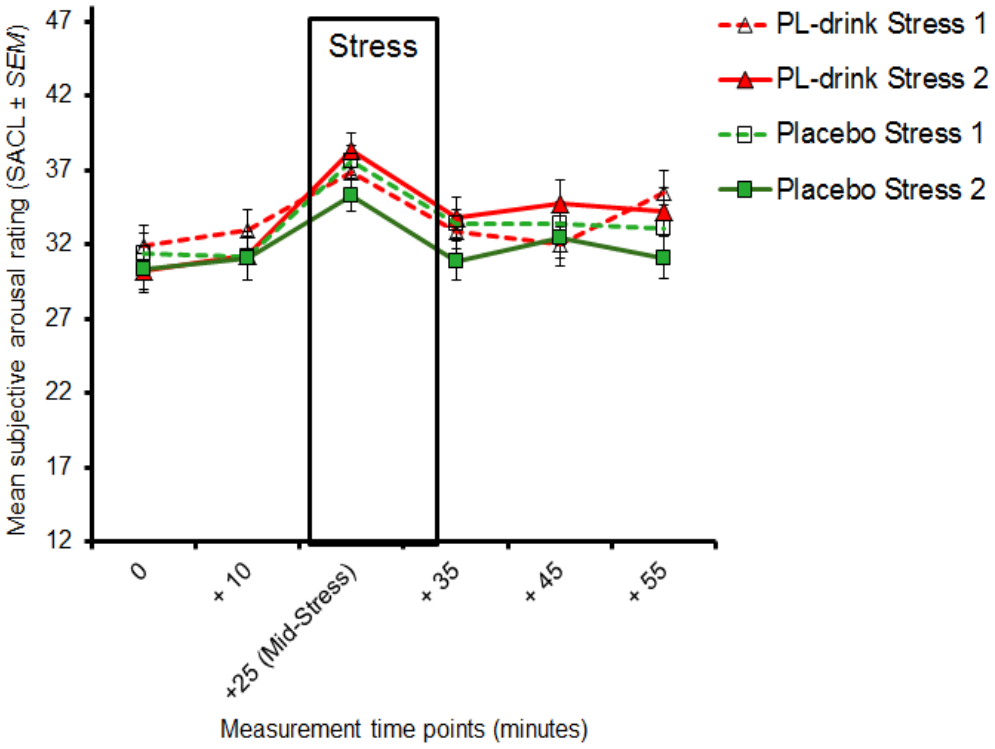


Figure 7.15 Mean (± *SEM)* subjective arousal rating (SACL) according to drink condition and stress visit

7.4.4.4 Effect of drink condition on subjective arousal (SACL)

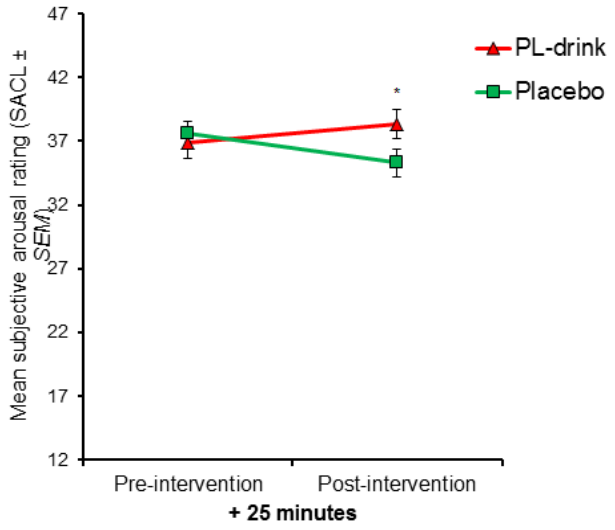
Mixed model ANCOVAs controlling for subjective arousal rating at Stress Visit 1 (baseline) revealed baseline subjective arousal was a significant predictor of subjective arousal across all measurement time points at Stress Visit 2 (*F* statistics are shown in Table 7.9).

Table 7.9 *F* statistics for relationship between subjective arousal rating (SACL) dimension ratings at Stress Visit 1 baseline (covariate) and ratings at Stress Visit 2 (post-intervention) at each measurement time point

Measurement time point (minutes)	Arousal (SACL)	
	F value*	*p*
0	13.60	< .001
+ 10	21.10	< .001
+ 25	26.81	< .001
+ 35	14.64	< .001
+ 45	9.69	< .003
+ 55	32.17	< .002

*df = 1,49

A significant main effect of drink condition, $F(1,50) = 7.49$, $p = .01$, was revealed for mid-stress subjective arousal ratings at + 25 minutes. Participants consuming the PL-drink reported significantly higher mid-stress subjective arousal ratings during Stress Visit 2 (significant at $p = .01$; see Figure 7.16).



* p = .01

Figure 7.16 Mean (± *SEM)* subjective arousal (SACL) pre + post intervention according to drink condition at + 25 minutes

7.4.4.5 Chronic stress (PSS)

A mixed model ANCOVA controlling for chronic stress rating (PSS) at Stress Visit 1 (baseline) found no significant differences between conditions for chronic stress rating

post- intervention (PL-drink, $\overline{X}$ = 14.07 ± 1.19; placebo, $\overline{X}$ = 15.19 ± 1.05), $F(1,49) = 0.02$, $p = .09$.

7.4.4.6 Profile of Mood States (POMS)

No significant effects were found for Confusion-bewilderment or Depression-dejection POMS dimensions.

7.4.4.7 Tension-anxiety response to stress (POMS)

A significant condition×time×visit interaction, $F(10,136) = 7.61$, $p < .001$, was found for the profiles of subjective Tension-anxiety mood ratings across the two stress visits (Figure 7.17). The significant condition×visit×time interaction reflected elevated anticipatory pre-stress Tension-anxiety mood ratings demonstrated in both drink conditions at Stress Visit 2. No significant differences in mean ratings were evident across the Stress Visit 2 profile in the PL-drink condition. However, a peak pre-stress rating at + 10 minutes was significantly higher than post-stress rating at + 55 minutes in the placebo condition. Heightened anticipatory Tension-anxiety ratings at + 10 minutes were significantly higher than the corresponding Stress Visit 1 ratings across both drink conditions ($p < .001$).

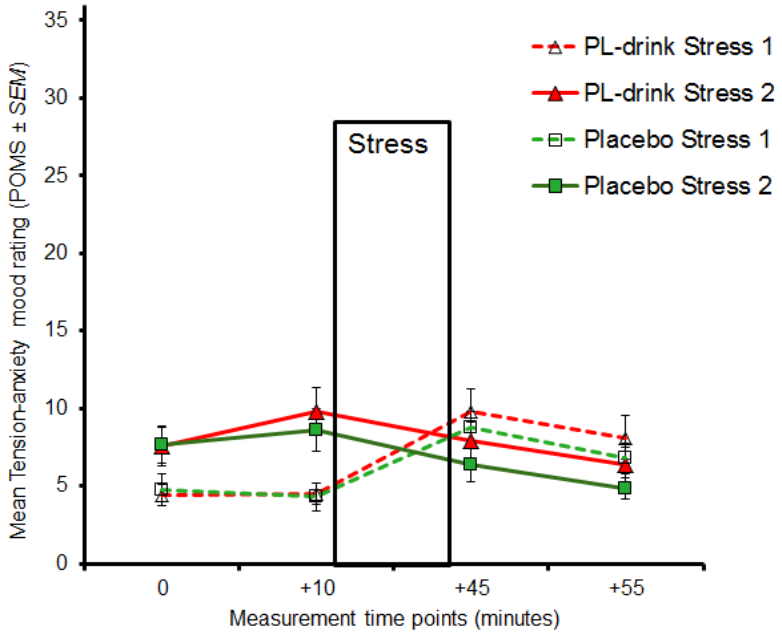


Figure 7.17 Mean (± *SEM)* subjective Tension-anxiety mood rating (POMS) according to drink condition and stress visit

There were also significant main effects of time, $F(3,156) = 6.32$, $p = .004$, and visit, $F(1,52) = 7.84$, $p = .007$, but no effect of drink condition, $F(1,51) = 0.36$, $p = .30$ (Figure 7.17). No significant differences were revealed across the subjective Tension-Anxiety response profile between drink conditions at Stress Visit 1 suggesting a comparable response kinetic across the drink conditions at baseline. Post-hoc comparisons revealed a post-stress peak Tension-anxiety mood rating at + 45 minutes during Stress Visit 1. For both drink conditions, the mean subjective Tension-anxiety rating at + 35 minutes was significantly higher than pre-stress ratings at 0 and + 10 minutes (both significant at $p < .001$).

7.4.4.8 Vigour-activity response to stress (POMS)

A significant condition×time×visit interaction, $F(10,136) = 5.13$, $p = .02$, was revealed for the profiles of subjective Vigour-activity mood ratings across the two stress visits (Figure 7.18). Post-hoc comparisons revealed no significant differences in subjective Vigour-activity response across the two stress visit profiles in either drink condition. The significant condition×time×visit interaction reflects participants in the placebo condition reporting significantly lower levels of subjective Vigour-activity prior to stress induction at Stress Visit 2. Mean Stress Visit 2 Vigour-activity ratings at 0 and + 10 minutes were significantly lower than corresponding ratings during Stress Visit 1 for this drink condition (both significant at $p < .02$). This significant attenuation of subjective Vigour-activity was not shown in the PL-drink condition. Post-stress, a decline in Vigour-arousal ratings was evident in the placebo condition by + 55 minutes, whilst post-stress response (+ 45 minutes) levels were maintained in the PL-drink condition.

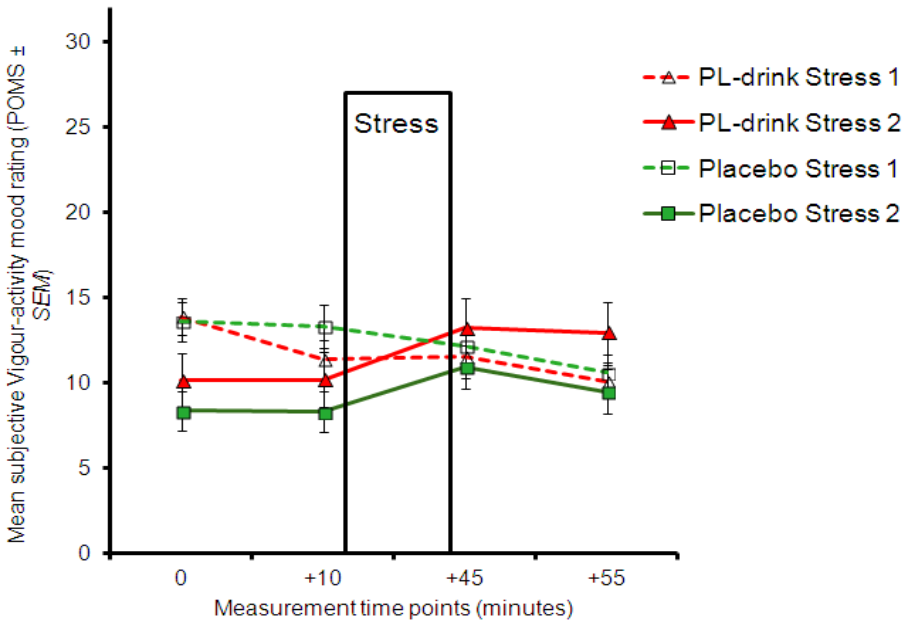


Figure 7.18 Mean (± *SEM)* subjective Vigour-activity mood rating (POMS) according to drink condition and stress visit

The main effect of visit, $F(1,52) = 18.44$, $p < .001$, was significant but effects of drink condition, $F(1,51) = 0.30$, $p = .60$, and time, $F(3,156) = 2.04$, $p = .11$, were non-significant.

7.4.4.9 Total Mood Disturbance response to stress (POMS)

A significant condition×time×visit interaction, $F(10,132) = 5.53$, $p < .001$, was revealed for the profiles of POMS TMD mood score across the two stress visits (Figure 7.19). No significant differences between the TMD response profiles were revealed at Stress Visit 1 suggesting a comparable response kinetic across the drink conditions at baseline. The significant condition×time×visit interaction reflects a post-stress increase in TMD score in both conditions at Stress Visit 1 that was not repeated at Stress Visit 2. TMD score was significantly higher post-stress at + 45 compared to + 10 minutes pre-stress in the placebo condition ($p = .04$). In the PL-drink condition, TMD score was significantly higher post-stress at + 45 minutes compared to 0 and + 10 minutes pre-stress (both significant at $p < .04$). No significant differences were revealed across the Stress Visit 2 response profiles for either drink condition.

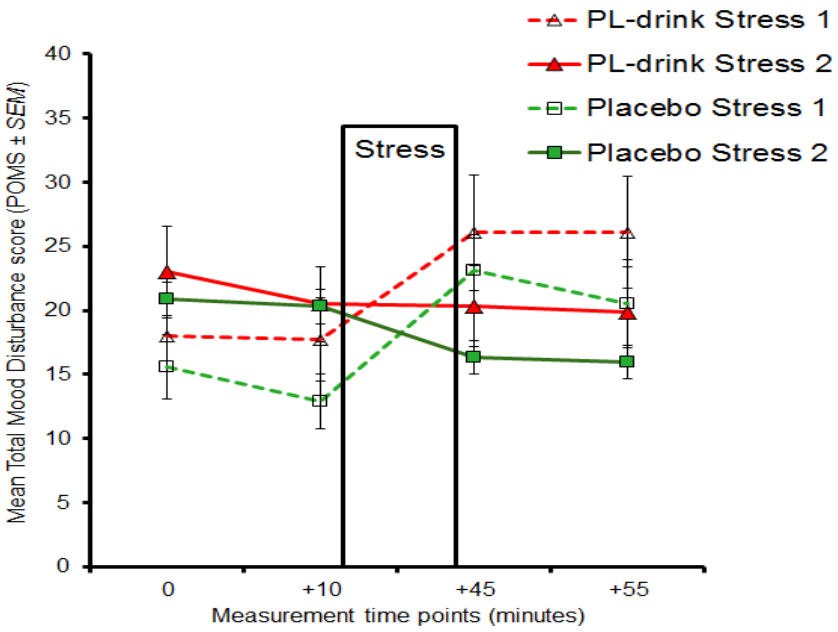


Figure 7.19 Mean (± *SEM)* Total Mood Disturbance score (POMS) according to drink condition and stress visit

No significant main effects of visit, $F(1,52) = 0.02$, $p = .35$, condition, $F(1,51) = 0.88$, $p = .83$, or time, $F(3,156) = 1.16$, $p = .33$, were found.

7.4.4.10 Anger-hostility response to stress (POMS)

A significant main effect of visit, $F(1,52) = 8.88$, $p = .04$, was revealed for Anger- hostility mood rating such that overall Anger-hostility was higher post-stress after Stress Visit 1 in both conditions. No significant effects of drink condition, $F(1,51) = 0.10$, $p = .75$, time, $F(3,156) = 1.87$, $p = .13$, or condition×time×visit interaction, $F(10,136) = 0.95$, $p = .48$, were found (*means* and *SD* for non-significant POMS dimensions are shown in Appendix 23).

7.4.4.11 Fatigue-inertia response to stress (POMS)

A significant main effect of visit, $F(1,52) = 3.79$, $p = .03$, and a marginally significant effect of time, $F(3,156) = 3.16$, $p = .06$, were revealed for Fatigue-inertia mood rating such that overall Fatigue-inertia ratings were higher pre-stress at Stress Visit 2 in both conditions. No significant effects of drink condition, $F(1,51) = 0.01$, $p = .91$, nor condition×time×visit interaction, $F(10,136) = 0.83$, $p = .59$, were found.

7.4.4.12 Effect of drink condition on POMS dimensions

Mixed models ANCOVAs controlling for subjective POMS dimension mood ratings at Stress Visit 1 (baseline) revealed baseline subjective Tension-anxiety, Vigour-activity, Confusion-bewilderment, Fatigue-inertia, and TMD POMS ratings were significantly related to corresponding ratings across all measurement time points at Stress Visit 2 (*F* statistics are shown in Table 7.10).

Table 7.10 *F* statistics for relationship between POMS dimension ratings at Stress Visit 1 baseline (covariate) and ratings at Stress Visit 2 (post-intervention) at each measurement time point

Measurement time point (minutes)	POMS dimension									
	Tension-anxiety		Vigour-activity		Confusion-bewilderment		Fatigue-inertia		TMD	
	*F*value*	*p*	*F*value*	*p*	*F*value*	*p*	*F*value*	*p*	*F*value*	*p*
0	14.30	< .001	43.74	< .001	20.50	< .001	16.81	< .001	38.52	< .001
+ 10	19.05	< .001	52.05	< .001	31.26	< .001	8.51	< .001	14.38	< .001
+ 45	37.16	< .001	39.15	< .001	32.59	< .001	15.00	< .001	43.70	< .001
+ 55	36.88	< .001	17,01	< .001	32.17	< .001	25.74	< .001	28.69	< .001

**df* = 1,47

TMD - Total Mood Disturbance

A significant main effect of drink condition was revealed for post-stress Vigour-activity rating at + 55 minutes, $F(1,46) = 4.13$, $p = .05$. Participants consuming the PL-drink reported significantly higher subjective Vigour-activity ratings at this time point during Stress Visit 2 (significant at $p < .05$; see Figure 7.20).

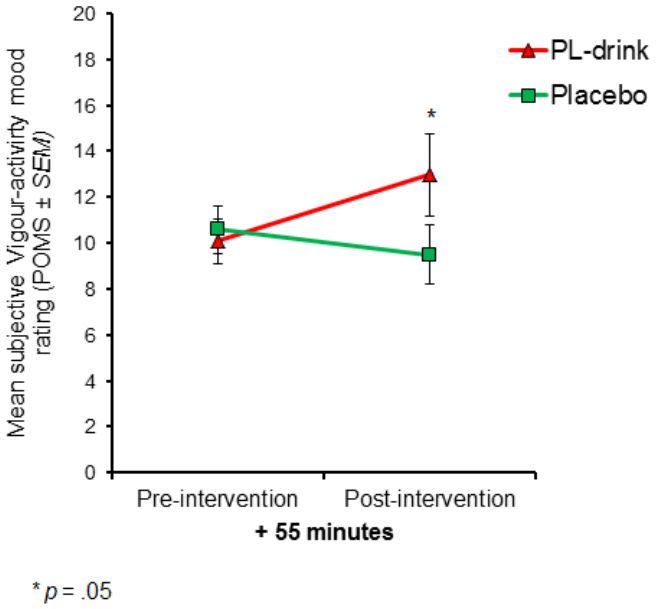


Figure 7.20 Mean (± *SEM)* subjective Vigour-activity rating *(*POMS) pre + post intervention according to drink condition at + 55 minutes

7.4.5 Relationships Between Subjective Stress and Physiological Stress Response Within Each Stress Visit

7.4.5.1 Stress Visit 1

7.4.5.1.1 PL-drink

Pearson's Product Moment (two-tailed) correlations revealed a significant relationship between subjective stress and SBP at +35, $r(26) = .56$, $p = .003$, and + 45, $r(26) = .48$, $p = .01$, minutes.

7.4.5.1.2 Placebo

Pearson's Product Moment (two-tailed) correlations revealed a significant relationship between subjective stress at + 25, $r(27) = .42$, $p = .03$, and + 35, $r(27) = .40$, $p = .04$, minutes and salivary cortisol at + 45 minutes.

7.4.5.2 Stress Visit 2

7.4.5.2.1 PL-drink

Pearson's Product Moment (two-tailed) correlations revealed a significant relationship between subjective stress and SBP at 0, $r(26) = .40$, $p = .04$, and + 10, $r(26) = .44$, $p =$

.03, minutes. Subjective stress at 0 minutes was also significantly related to salivary cortisol at 0, $r(26) = .50$, $p = .01$, and + 25, $r(26) = .34$, $p = .05$, minutes.

7.4.5.2.2 Placebo

Pearson's Product Moment (two-tailed) correlations revealed a significant relationship between salivary cortisol at + 10 minutes and subjective stress at + 25, $r(27) = .42$, $p = .03$, minutes.

7.4.6 Cognitive Performance

7.4.6.1 2-back

One participant from the placebo condition was removed from 2-back data analysis due to demonstration of performance > 3.8 SD below the sample mean across all 2-back outcome measures (baseline and post-intervention).

Mixed models ANCOVAs controlling for performance at Stress Visit 1 (baseline) revealed baseline 2-back performance on each respective 2-back outcome variable was a significant predictor of 2-back target accuracy, $F(1,48) = 102.96$, $p < .001$, total accuracy, $F(1,48) = 51.06$, $p < .001$, target RT, $F(1,48) = 41.43$, $p < .001$, and non-target RT, $F(1,48) = 83.19$, $p < .001$, post drink at Stress Visit 2.

A significant main effect of drink condition, $F(1,48) = 10.16$, $p = .003$, and a significant baseline×condition interaction, $F(1,48) = 9.38$, $p = .004$, were revealed for target accuracy. Post-hoc comparisons revealed no significant differences in performance post-intervention across drink conditions ($p = .21$; see Figure 7.21). A pattern of higher baseline accuracy performance in the PL-drink condition was maintained pre- and post-intervention.

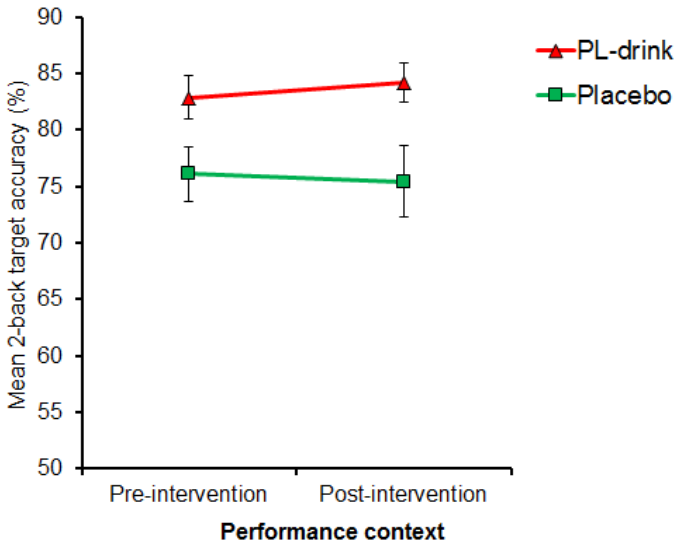


Figure 7.21 Mean (± *SEM)* 2-back target accuracy (%) performance according to drink condition pre + post intervention

No significant main effects of drink condition or significant baseline×condition interactions were revealed for 2-back total accuracy, target RT, or non-target RT.

7.4.6.2 A-ospan

Mixed models ANCOVAs controlling for performance at Stress Visit 1 (baseline) revealed no significant main effects or interactions for A-ospan performance outcome measures (see Table 7.11). A pattern of improved performance in the placebo condition was demonstrated post-intervention (see Figure 7.22). This can be considered to be a function of the lower baseline performance level in participants randomised to this condition. Participants in the PL-drink condition were performing close to ceiling (A-ospan score range = 0 – 60) at baseline so had less room for improvement. The lower baseline performance levels in placebo condition participants allowed for greater scope for improved performance at Stress Visit 2.

Table 7.11 *F* statistics for A-ospan performance outcome measures controlling for Stress Visit 1 baseline (covariate)

A-ospan performance outcome measure	Main effects and interactions					
	Baseline		Condition		Baseline×Condition	
	F value*	*p*	*F* value*	*p*	*F* value*	*p*
Speed accuracy	0.52	.48	0.33	.57	0.01	.93
Score	2.32	13	0.10	.75	0.16	.70
Total	0.69	.41	2.2	.14	2.36	.13
Maths error	0.56	.39	0.68	.41	1.03	.32

* *df* = 1,49

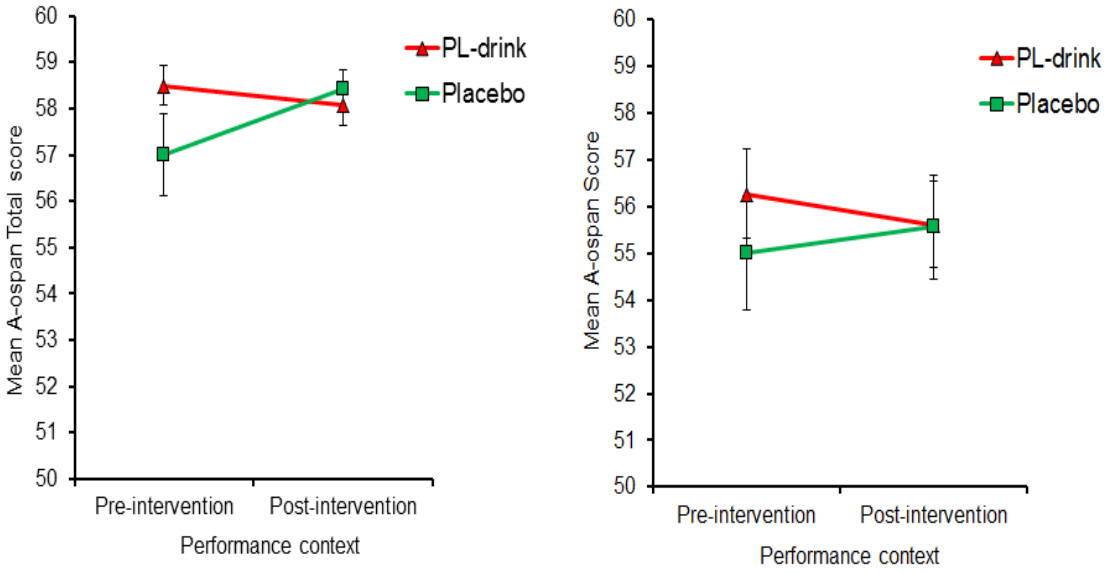


Figure 7.22 Mean (± *SEM)* A-ospan Score (right panel) and Total performance (left panel) according to drink condition pre and post intervention

7.4.6.3 Task-switch test

One participant from the placebo condition was removed from task-switch test analysis due to performance > 4 SD below the sample mean across all task-switch test outcome measures (baseline and post-intervention). Mixed models were employed to examine performance between task-switch trial types and switch costs. Mixed models ANCOVAs controlling for baseline were employed to examine whether performance across these outcome measures differed between drink conditions post-intervention (Stress Visit 2).

7.4.6.4 Effect of stress on task-switch accuracy

A significant main effect of visit, $F(1,51) = 20.01$, $p < .001$, and trial, $F(2,102) = 33.08$, $p < .001$, were revealed across trial accuracy performance. No effect of drink condition was found, $F(1,50) = 2.41$, $p = .13$ (see Figure 7.23).

During Stress Visit 1 (baseline), switch trial accuracy was significantly lower across both drink conditions compared to repeated nested and pre-switch trials (all significant at $p < .04$). During Stress Visit 2 (post-drink), switch trial accuracy was significantly lower than nested and pre-switch trials only in the placebo condition (both significant at $p < .02$).

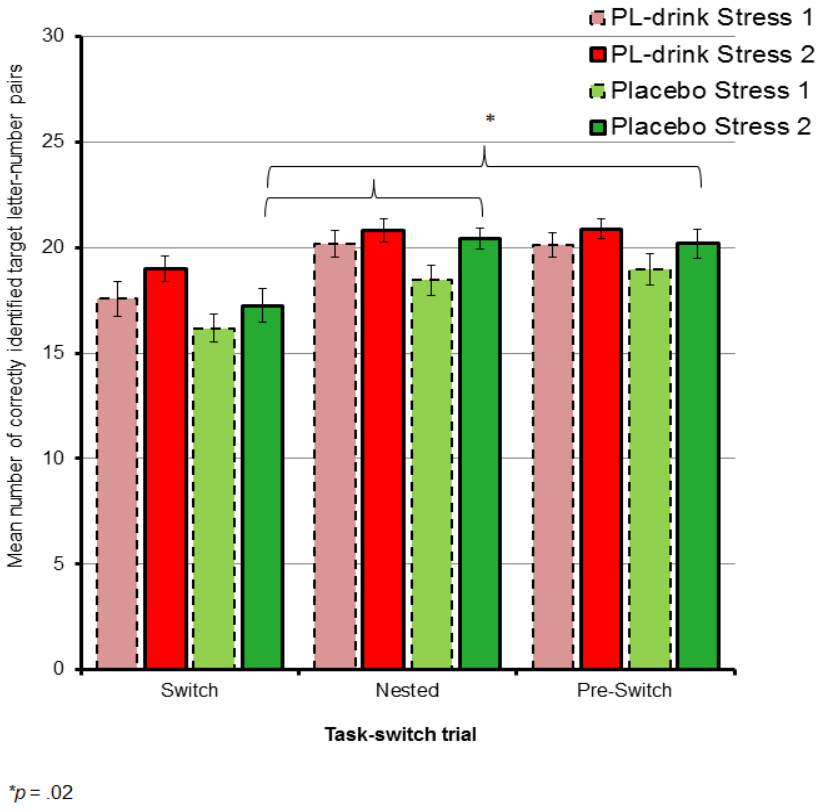


Figure 7.23 Mean (± *SEM)* number of correctly identified target letter-number pairs across task-switch trials pre + post intervention

7.4.6.5 Effect of drink condition on task-switch accuracy

Mixed models ANCOVAs controlling for performance at Stress Visit 1 (baseline) revealed baseline target accuracy was a significant predictor of target accuracy across all trial types at Stress Visit 2: switch, $F(1,49) = 9.71$, $p = .003$; nested, $F(1,49) = 5.52$, $p = .02$; pre-switch, $F(1,49) = 12.76$, $p < .001$.

A significant main effect of drink condition, $F(1,49) = 8.71$, $p = .01$, and a baseline×condition interaction, $F(1,49) = 7.26$, $p = .01$, were revealed for switch trial accuracy. A trend for increased post-intervention accuracy in both drink conditions was demonstrated. However, post-hoc comparisons revealed no significant differences in switch trial accuracy between drink conditions at Stress Visit 2 ($p = .17$).

7.4.6.6 Effect of stress on task-switch RT

A significant main effect of trial, $F(2,102) = 106.98$, $p < .001$; Figure 7.24) was revealed across trial RT performance. Reaction time (ms) for the identification of target letter-number pairs during switch trials was significantly slower than repeated nested and pre-switch trials across both drink conditions pre- and post-intervention (all significant at $p < .001$).

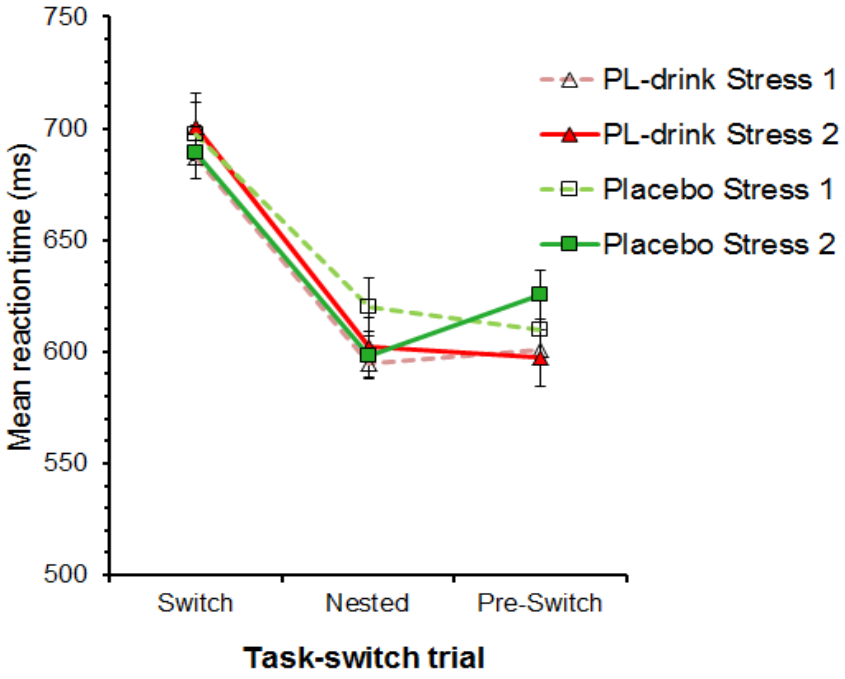


Figure 7.24 Mean (± *SEM)* RT (ms) across task-switch trials pre + post intervention

7.4.6.7 Effect of drink condition on task-switch RT

Mixed models ANCOVAs controlling for performance at Stress Visit 1 (baseline) revealed baseline RT was a significant predictor of RT on switch, $F(1,49) = 6.68$, $p = .01$, nested $F(1,49) = 6.19$, $p = .02$, and pre-switch trials, $F(1,49) = 16.09$, $p < .001$, post-intervention at Stress Visit 2.

A significant main effect of drink condition, $F(1,49) = 5.35$, $p = .01$, and a significant baseline×condition interaction, $F(1,49) = 5.72$, $p = .01$ were revealed for nested trial RT. A significant main effect of drink condition, $F(1,49) = 10.14$, $p = .002$, and a significant baseline×condition interaction, $F(1,49) = 9.15$, $p = .004$, were also revealed for pre-switch trial RT. However, post-hoc comparisons revealed no significant trial RT differences between drink conditions at Stress Visit 2 for either switch trial (see Figure 7.25).

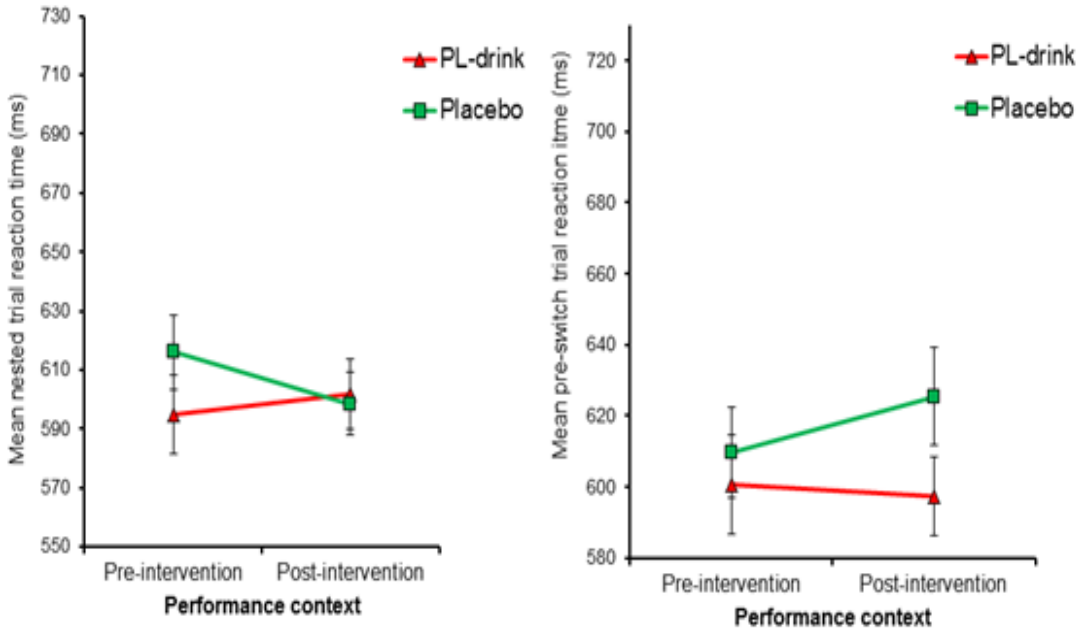


Figure 7.25 Mean (± *SEM)* nested (left panel) and pre-switch (right panel) RT across condition pre and post intervention

7.4.6.8 Effect of stress on accuracy and RT switch costs

A significant main effect of trial (switch/repeat), $F(1.51) = 35.69$, $p < .001$, was revealed for accuracy switch cost. The accuracy switch cost (performance decrement) incurred during switch trials was significantly higher than the switch cost incurred during repeat trials across both drink conditions pre- and post-intervention (all significant at $p < .001$; see Figure 7.26).

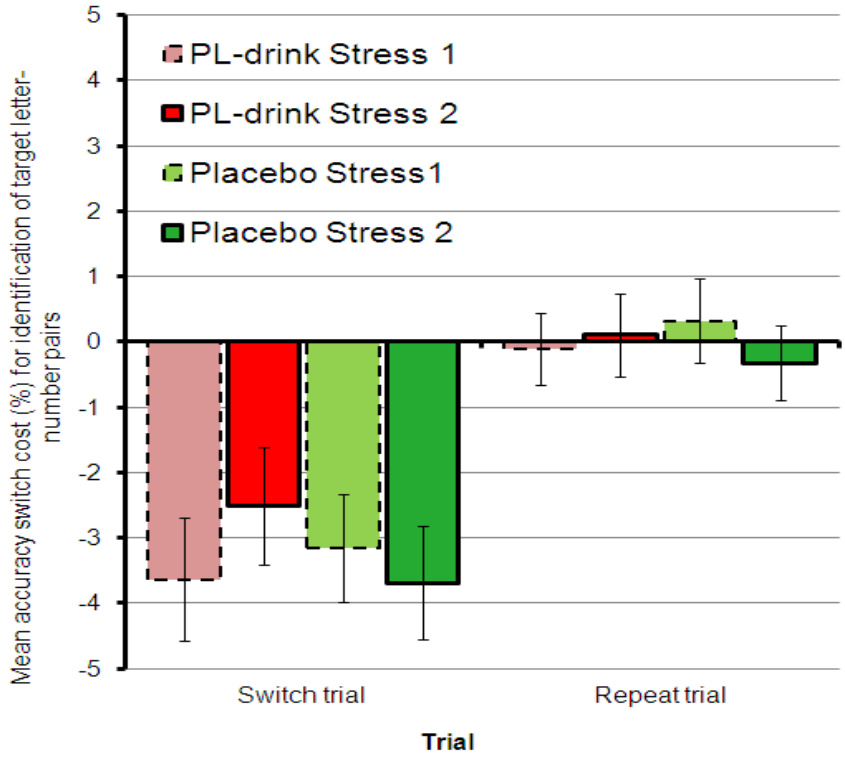


Figure 7.26 Mean (± *SEM)* performance accuracy (%) switch cost across switch and repeat trials pre + post intervention

A significant main effect of trial was also revealed for RT switch cost, $F(1,51) = 122.04$, $p < .001$. The RT switch cost (performance decrement) incurred during switch trials was significantly higher than the switch cost incurred during repeat trials across both drink conditions pre- and post-intervention (all significant at $p < .001$; see Figure 7.27).

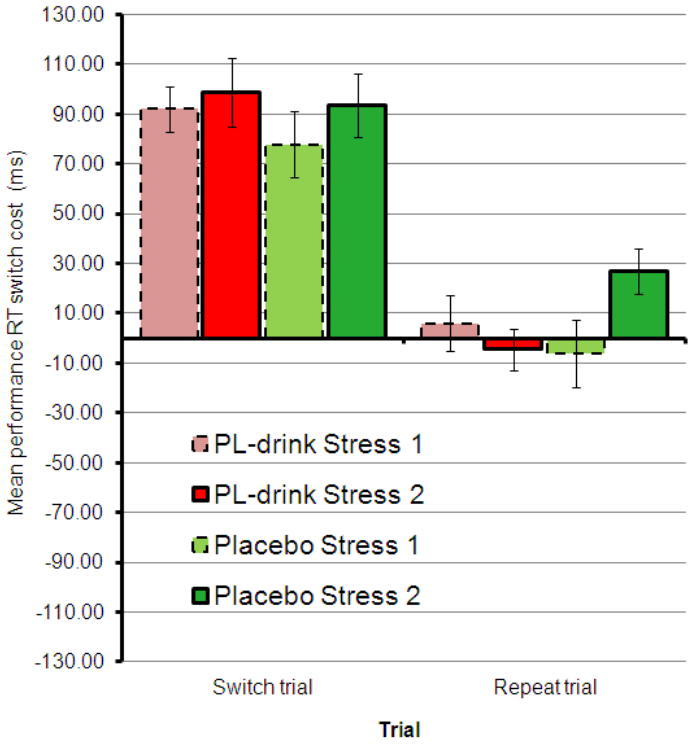


Figure 7.27 Mean (± *SEM)* RT switch cost across-switch and repeat trials pre + post intervention

7.4.6.9 Effect of drink condition on accuracy and RT switch costs

Mixed models ANCOVAs controlling for performance at Stress Visit 1 (baseline) revealed a significant main effect of drink condition on switch cost RT on repeat trials, $F(1,48) = 6.66$, $p = .01$. Post-hoc comparisons revealed the RT switch cost (performance decrement) incurred during switch trials was significantly higher for participants in the placebo condition (see Figure 7.28). Placebo participants incurred significantly higher switch costs on repeat trials than PL participants ($p = .01$).

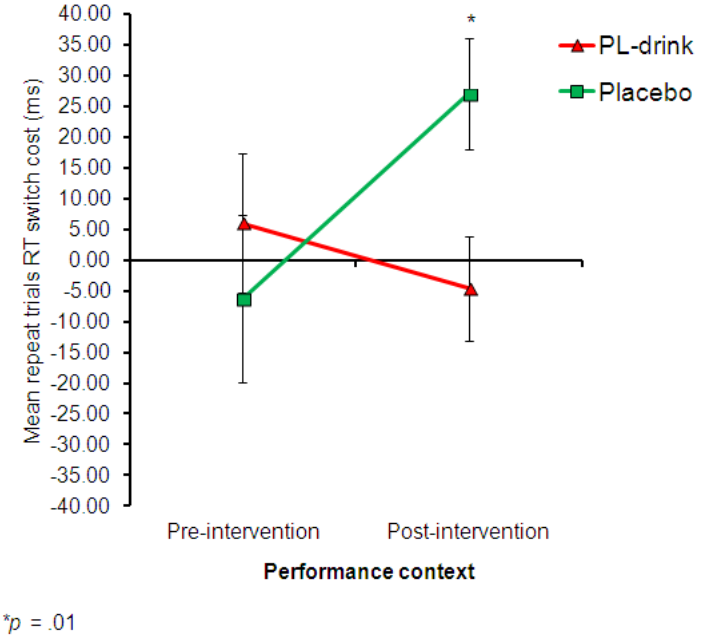


Figure 7.28 Mean (± *SEM)* RT switch cost for repeat task-switch trials pre + post intervention

7.4.7 Cognitive Distraction/Interference and Cognitive Performance

No significant effects of visit or condition were revealed for general or stress context-related cognitive distraction/interference VAS. Pearson's Product Moment (two-tailed) product moment correlations revealed no significant correlations between cognitive distraction/interference VAS ratings and cognitive performance outcome measures at Stress Visit 1 or Stress Visit 2.

7.5 Discussion

7.5.1 Salivary Cortisol Response

Contrary to the hypothesised attenuation of salivary cortisol response by PL intake, a trend for an increased response was demonstrated post-intervention across the profile. A tendency towards increased cortisol responsivity and CAR after administration of PLs has been previously demonstrated in high stress-load men (Schubert et al., 2011). However, the finding contradict previous evidence of the potential of PL to attenuate cortisol

responses to stress (Fahey & Pearl, 1998; Hellhammer et al., 2004; Monteleone et al., 1990; Monteleone et al., 1992; Starks et al., 2008). It is worth noting that these studies differed in terms of PL source (bovine cortex or soy) and stressor (predominantly exercise). An increase in anticipatory cortisol response in the PL condition was significantly elevated compared to pre-intervention levels. This response sensitisation was also demonstrated to a lesser extent in the placebo condition. Trends towards elevated anticipatory cortisol responses following PL intake have also been reported previously (Hellhammer et al., 2010; Schubert et al., 2011). The heightened anticipatory response also appears to be a characteristic of repeated exposure to a stressor independent of dietary intervention (demonstrated in Study 3 and Kirschbaum et al., 1995; Wust et al., 2005). Phospholipid intake may have served to increase the level of this expected response to some degree. However, no significant differences between the drink conditions were revealed across the response profiles once baseline levels had been accounted for; only baseline cortisol levels significantly predicted post-drink intervention response.

Comparable cortisol AUCg between drink conditions and across stress visits suggests no significant effects of drink or repeated visit on absolute net response. Differences emerged within the aggregated measures of responsivity. The significant decrease in AUCi and delta increase in the PL-drink condition suggests a significant response reduction in this drink condition. The fact that a main effect of visit but not condition was reported may suggest this difference was a result of habituation in response rather than an observed effect of drink condition. However, inspection of the response profile reveals that this significantly reduced response at stress visit two was reflective of anticipatory response sensitisation. Indeed, the post-stress response trajectory was analogous to the response elicited at Stress Visit 1.

High chronic stress was identified as an exclusion criterion due to associations with attenuation of cortisol responsivity (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Hellhammer & Wade, 1993) and evidence of higher cortisol responsivity in low chronic stress groups (Hellhammer et al., 2012). Participants in the PL-drink condition demonstrated a higher, but non-significant, level of chronic stress at study entry. Chronic stress in this condition was marginally below a score of 15 on the TICS CSSS scale. This cut-off point was adopted by Schubert et al. (2011) to distinguish between high and low stress-load. A sample of men scoring ≥ 15 on the TICS demonstrated a similar tendency for elevated cortisol following PL intake over the same intervention period. Therefore, the level of stress-load at study entry may have contributed to the effects demonstrated.

The aim of examining the potential of PL intake to moderate cognitive performance in a stress context that elicited comparable response over repeated exposures was identified as an objective of this thesis. A tendency toward increased anticipatory but comparable peak and post-stress response trajectory was demonstrated in the piloting of this laboratory stress protocol in Study 3. A comparable response kinetic was demonstrated here. Despite heightened anticipatory responses in both drink conditions, a comparable post-stress response, temporally corresponding to completion of cognitive tests, was demonstrated within each condition. Despite random allocation to drink conditions the tendency toward a greater post-stress response trajectory was demonstrated by participants randomised to the PL condition pre- and post-intervention. Higher cortisol responses have been associated with more pronounced habituation to repeated stress (Hellhammer et al., 2012; Schommer et al., 2003; Wust et al., 2005b). Rather, a sensitisation in pre-stress levels and comparable peak and post-stress response trajectory was demonstrated here.

The relative contribution of selecting participants high in perfectionism to the cortisol responses exhibited was not possible without the inclusion of a low perfectionism comparator group. Whilst this would have given a valuable insight into the hypothesis developed across the thesis that perfectionism is related to cortisol responsivity, the primary aim of the intervention study was the examination of the potential for PL intake to moderate stress responses and cognitive performance. However, on average the pre-intervention salivary cortisol delta increase in participants high in perfectionism (whole sample) recruited into Study 4 (X = 7.98 ± 0.85 nmol/L) exceeded that of the sample recruited in Study 3 (X = 5.86 ± 1.22 nmol/L). Further examination of the potential role of perfectionism in distinguishing between responder types is warranted.

7.5.2 Cognitive Performance

Contrary to hypothesised effects, PL intake failed to moderate WM performance under stress. Evidence of significantly higher RT switch costs in the placebo condition compared to the PL condition was demonstrated. However, the importance of this effect is debatable considering performance on the task-switch test as a whole. The expected increased RT on switch vs. repeat trials was consistently demonstrated by both drink conditions. Placebo condition participants demonstrated an apparent trend for slower RT on repeat nested and pre-switch trials at baseline (Stress Visit 1). This slower RT was maintained for pre-switch trials post-intervention. This consistent trend for slower RT performance in this condition likely accounts for the higher RT switch costs post-

intervention rather than a specific effect of drink condition. By comparison, PL participants demonstrated consistent RT switch costs pre- and post-intervention. Moreover, the impairing effect of stress on this cognitive test primarily relates to the further detriment of impaired performance when switching between task-sets. Switching between task-sets (switch vs. repeat trials) incurs a performance cost; namely reduced accuracy and RT when switching between task-sets. This is a well characterised effect under normal conditions (Monsell, 2003; Wylie et al., 2003). Stress has been demonstrated to augment this effect (Plessow et al., 2012). It is on this performance impairment that protective effects of an intervention would be expected to act. However, the expected impaired switch cost between switch and repeat trials was consistently demonstrated by both drink conditions for both switch cost accuracy and RT. Therefore, the observed difference between repeat trial switch cost between condition was considered a nominal effect likely reflective of divergent aptitude on this test. Evidence of impaired post-intervention trial accuracy demonstrated in the placebo condition further reflects this. Despite a comparable pattern of lower switch trial accuracy in both conditions compared to repeat nested and pre-switch trials, this performance detriment was only significantly maintained pre- and post-intervention in the placebo condition. Rather than an effect of drink condition, this reflects consistently lower performance accuracy on switch trials in the placebo condition.

Such findings highlight the difficulty interpreting cognitive data that is undermined by consistent divergence in cognitive performance level at baseline. Participants randomised to the PL condition demonstrated superior cognitive performance across all outcomes pre-intervention. Whilst analysis controlling for baseline performance, rather than performance percentage change from baseline, accounts for this issue to an extent, results from samples with unequal baseline performance (between conditions) need to be treated with caution. For example, compared to placebo participants, PL participants were performing closer to ceiling on the A-ospan task pre-intervention. This reduced the potential for improvement in the PL condition, whilst scope for improvement was more possible for the participants randomised to the placebo condition.

The divergence in performance level at baseline occurred despite random allocation of individuals to drink conditions. Pronounced inter-individual differences in WM performance, most likely underpinned by factors such as differential attentional process capabilities and fluid intelligence (Awh, Vogel, & Oh, 2006; Unsworth & Engle, 2005), have been reported (Schoofs, Pabst, Brand, & Wolf, 2013; Schoofs et al., 2008). Such differences provide the main rationale for random allocation of participants to conditions.

Individuals did not significantly differ across age, BMI, chronic stress level, or perfectionism at study entry. A difference in HADS depression score was considered negligible considering the low score in both conditions. A trend for higher peak cortisol response was demonstrated in the PL condition but higher cortisol responses have previously been associated with lower rather than the higher performance level on the types of cognitive tests administered here. Moreover, a trend for higher cognitive performance, albeit less pronounced, in PL participants was demonstrated during screening practice trials performed in the absence of stress. This suggests randomisation failed to adequately partition individuals of varying ability across the two conditions. The quasi-random allocation of participants to condition following an initial assessment of performance level is a potential method of reducing the probability of this confounding effect and should be considered in future studies.

7.5.3 Cardiovascular Response

An unexpected effect of PL intake was increased cardiovascular response. This increased responsivity was evident both in anticipatory (0 minutes [SBP]) and response recovery (+ 35 [SBP] and + 45 [DBP, SBP by trend]). In contrast to cognitive effects, this post-intervention differentiation in response was not associated with a divergence in response at baseline. No moderation of cardiovascular parameters by PL intake has been reported in previous stress induction studies (Hellhammer et al., 2004; Hellhammer et al., 2012; Monteleone et al., 1990; Monteleone et al., 1992; Parker et al., 2011). Indeed, PL intake has previously been associated with reduced basal BP (Richter, Herzog, Lifshitz, Hayun, & Zchut, 2013) and positive moderation of markers related to cardiovascular function (e.g., lowered blood cholesterol; Kuellenberg et al., 2012). No significant differences in cold pressor hand submersion were demonstrated so this cannot account for the differences in cardiovascular tone.

One potential mechanism for this effect on blood pressure is the fatty acid composition of bovine milk PLs. A number of fatty acids associated with bovine milk PLs have been correlated with elevated BP in hyper- and normotensive adults. For example, saturated palmitic acid has been associated with a small increases in basal SBP in a cross-sectional plasma lipid analysis of healthy men (Grimsgaard, Bonaa, Jacobsen, & Bjerve, 1999; Simon, Fong, & Bernert, 1996). However, saturated fatty acids associated with milk PLs have also been correlated with reduced BP (stearic acid; Simon et al., 1996). Moreover, unsaturated fatty acids (e.g., linoleic and oleic acid) which are more densely represented in the PL pool are associated with reduced basal BP (Grimsgaard et al.,

1999; Miura et al., 2008; Simon et al., 1996). The findings of such studies likely reflect the accumulated effects of dietary intake over longer periods of time than the intervention study reported here, and report basal rather than reactive BP. Conclusions are further complicated by the fatty acid composition of bovine milk being highly complex and varied as a function of factors such as genetics (breed and selection), seasonal and regional factors, feed, and stage of lactation (Palmquist, Beaulieu, & Barbano, 1993). Without composition analysis of the specific fatty acid pool of the intervention drinks and subsequent effect on serum fatty acid composition, comparisons with such results are speculatory.

Finally, the permissive effects of cortisol on the cardiovascular response to stress may have contributed to the divergent post-intervention BP response. The permissive effects of GCs on BP and cardiac output have been demonstrated in humans and animal models (Sapolsky et al., 2000). In most cases (predator avoidance being one exception), GCs act to 'permit' catecholamines and other vasoconstrictors to exert their full actions by augmenting cardiovascular activation during stress (Krakoff, 1988). Mechanisms include a positive inotropic effect on vascular and cardiac tissues (Sambhi, Weil, & Udhoji, 1965), the inhibition of catecholamine reuptake and peripheral catechol-*O*-methyltransferase and monoamine oxidase (catecholamine degrading enzymes; Gibson, 1981; Kennedy & Ziegler, 1991), and increased cardiovascular sensitivity to catecholamines (Sapolsky et al., 2000). It may be hypothesised that the higher cortisol response demonstrated by participants in the PL condition may have augmented the cardiovascular response in this condition compared to the placebo.

7.5.4 Subjective Stress Responses

A pattern of heightened anticipation and attenuated mid-stress peak was demonstrated post-intervention under both drink conditions. Participants were not fully aware of the exact nature of the stress protocol at Stress Visit 1. A heightened anticipatory response seems inevitable upon repetition of stress exposure due to increased negative expectancies associated with previous experience of the stressful context. Drink condition was shown to moderate this anticipatory subjective stress response with a trend for attenuated anticipatory elevation in stress following PL intake. Conversely, participants consuming the placebo demonstrated elevated responses to the extent that stress ratings peaked prior to stress exposure. The potential for PLs to moderate subjective states has been previously reported; both increased subjective well-being/mood (Benton et al., 2001; Fahey & Pearl, 1998), and reduced stress perception (Hellhammer et al., 2004;

Hellhammer et al., 2010). The mechanism underpinning such effects is unclear. Baumeister et al. (2008) have previously demonstrated PL supplementation moderated neural spectral activity to induce an increased relaxed state. However, an increased relaxed state would be a difficult assumption to defend in this instance considering the accompanying heightened markers of stress in the PL condition. Moreover, subjective stress ratings were positively associated with heightened anticipatory SBP in this condition. Cortisol has been previously associated with reduced negative mood and lower levels of anxiety (Serkan, Het & Wolf, 2007; Schlotz et al., 2008). Therefore, higher cortisol response in the PL condition may explain this attenuated subjective response.

Significant correlations between subjective stress and cardiovascular (SBP) response, post-stress exposure (Stress Visit 1) and in anticipation of stress exposure (Stress Visit 2), were demonstrated by the PL-drink participants. No such correlations were revealed in the placebo condition. Accurate subjective appraisal of autonomic arousal appears to be limited (Baumann & Leventhal, 1985; Brondolo, Rosen, Kostis, & Schwartz, 1999), and variable between individuals (Cameron, 2001; Leopold & Schandry, 2001; Wiens, Mezzacappa, & Katkin, 2000). The increased correspondence between physiological and subjective stress responses may be suggestive of greater stress reactivity associated with heightened perception of stress activation in the PL condition. Indeed, heightened interoceptive sensitivity – the conscious perception of proprioceptive (e.g., muscle contraction) and viscероceptive (e.g., HR, BP and respiration) bodily signals – has been associated with the pathogenesis and maintenance of state and trait anxiety and anxiety-related disorders (Cameron, 2001; Domschke, Stevens, Pfleiderer, & Gerlach, 2010). However, high interoceptive sensitivity has also been associated with reduced state anxiety in anticipation of, and post, TSST exposure (Werner, Duschek, Mattern, & Schandry, 2009).

In accordance with increased sympathetic arousal in participants randomised to the PL-drink, subjective arousal was also significantly elevated compared to those allocated to the placebo drink. Evidence of heightened arousal mid-stress (SACL) and prolonged arousal during recovery (POMS [+ 55 minutes]) was revealed. This significant post-intervention increase in subjective markers of arousal in PL participants was demonstrated despite the lack of significant elevations in subjective arousal during the initial stress exposure (Stress Visit 1). Whilst the evidence of increased physiological and subjective arousal suggests the PL-drink induced a general state of enhanced arousal, no significant correlations emerged between the physiological and subjective arousal markers. This may be reflective of the measure of arousal employed here (SACL) being

primarily measures of energetic arousal. More consistent correlations may have been demonstrated if measures of tense arousal, which have been shown to be correlated with and moderated by cortisol response (Schlotz et al., 2008), were recorded.

In agreement with the findings of Hellhammer and Schubert (2012), the inclusion of a mid-stress rating ensured a more accurate characterisation of the subjective stress response. An important distinction between the stress visit response profiles would have been missed in the absence of a mid-stress rating; only the heightened anticipatory response, and not the mid-stress response habituation, would have been characterised. This has implications for previous studies in the thesis. The peak subjective stress response pre- and post- audition may have been subordinate to an even greater mid-audition peak. Similarly, the lack of apparent habituation in subjective stress exhibited in the repeated laboratory study may have occurred mid-stress.

7.6 Summary

Contrary to hypothesised effects, PL intake failed to attenuate the acute endocrine stress response and protect cognitive performance under conditions of stress. Rather, the intake of a bovine milk-derived PL drink tended to increase the salivary cortisol response to stress provocation and was associated with significantly increased physiological and subjective markers of autonomic arousal. In contrast to endocrine and physiological stress parameters, anticipatory subjective stress was attenuated by PL intake. Cognitive performance was unaffected by dietary manipulation. Furthermore, the examination of the effects of PL intake on cognitive performance under conditions of stress was undermined by a divergence in cognitive test aptitude between the groups of participants randomised to the experimental drink conditions at baseline. Conclusions on the potential for dietary PLs to moderate cognitive performance and stress responses under stressful conditions are therefore compromised by a number of underlying differences in individuals randomised to the drink conditions. Nonetheless, the findings from Study 4 do not support the potential for PL intake to positively moderate stress responses or to proffer protective effects on cognitive performance tests demonstrated to be sensitive to stress provocation.

Chapter 8:
General Discussion

Chapter 8 General Discussion

8.1 Overview of the Thesis

The findings of the thesis will be discussed in further detail below in relation to the overall thesis aims:

I. Explore the use of a real-world or laboratory stressor for a dietary intervention study

II. Assess the capability of the identified stress context to elicit comparable stress (primarily cortisol) responses over repeated exposures

III. Identify cognitive tests sensitive to the impairing effects of stress

IV. Identify potential predictors of cortisol responsivity for selection of an intervention sample likely to elicit robust cortisol responses

V. Examine the effects of a PL intervention on cognitive performance and stress responses under conditions of stress

The potential for PLs to moderate cognitive performance and endocrine and psychological stress responses has been inconsistently reported in the literature. Early research demonstrating that PLs could attenuate cortisol responses led to research examining the potential for these lipids to offer protective effects from the impairing effects of stress. The psychoneuroendocrine stress response systems instigate a wide array of permissive and suppressive responses to cope with the challenge of stress. Cognition is one function which has been demonstrated to be affected by stress provocation. The primary role of GCs (cortisol in humans) has been emphasised in this moderation of cognitive performance. Therefore, evidence of the potential for PLs to attenuate the cortisol response suggests intake of these lipids may offer functional benefits for performance under conditions of stress.

To date, the evidence for this effect has been mixed and inconsistent. Early evidence of the attenuation of cortisol responses by bovine cortex PS (Monteleone et al., 1990; Monteleone et al., 1992) and soy-derived PLs (Hellhammer et al., 2004) showed significant attenuation in cortisol responsivity. Later research employing soy and bovine milk-derived PLs has demonstrated inconsistent and small effects on cortisol response to stress (e.g., Hellhammer et al., 2010; Parker et al., 2011). This moderation of cortisol may operate as a function of underlying stress vulnerability with stress-load and cortisol responsivity moderating the potential for PL supplementation to dampen (or augment)

stress responses (Hellhammer et al., 2012; Schubert et al., 2011). The potential for PL intake to protect cognitive performance under conditions of stress has received even less consistent support. This may be due to the lack of significant attenuation of cortisol responses by PL intake in studies assessing cognitive function. Furthermore, the limited evidence of protective effects of PL intake on cognitive function may be due to the sensitivity of the specific tests employed. Evidence from the general stress and cognition literature demonstrates variable impairment of cognitive performance by stress provocation. This is, in part, underpinned by the divergent effects of stress on specific cognitive domains and cognitive processes, and by the specific sensitivity of particular tests of cognitive performance used to measure change in performance level. Moreover, significant impairment of cognitive functions may only be demonstrated by individuals demonstrating large cortisol and sympathetic stress responses (Buchanan & Tranel, 2008; Buchanan et al., 2006; Elzinga & Roelofs, 2005; Takahashi et al., 2004).

Evidence from the existing literature and work undertaken during the thesis were used to plan an intervention study to further explore the potential for PL intake to moderate cognitive performance under acute stress. The thesis culminated in a study examining cognitive performance and stress response parameters before and after a six week intake of a PL-rich or placebo drink. Three studies were undertaken prior to this PL intervention study with the aim of identifying appropriate design and methodological approaches to employ.

8.1.1 Identification of a Stress Context

Studies 1 and 2 focussed on examining the potential for employing a naturalistic real-world stress context in the intervention study. This was considered potentially advantageous since there is a paucity of research examining the effects of PL intake in naturalistic contexts characterised by genuine social-evaluative threat. Furthermore, there is some evidence to suggest naturalistic competitive performance stress contexts may be less prone to significant habituation in HPA-mediated stress responses (Rohleder et al., 2007). The decision to expose participants to repeated stress in a within-subjects manner in the intervention study meant the identification of a stressor capable of eliciting comparable and significant cortisol responses over repeated exposures was required. Whilst a competitive performance audition context was sufficient to elicit significant endocrine, cardiovascular, and subjective stress responses in Study 1, this effect was not replicated in Study 2. Furthermore, difficulty categorising participants into groups based on previous level of audition experience (Study 1), and the decision to not conduct a

repeated audition stress visit (Study 2) resulted in a failure to characterise the level of habituation in this context. Therefore, a laboratory stress context was employed for Study 3 to increase the levels of control and standardisation. The decision to recruit a male only sample for Study 3 and 4 was one measure adopted to control for variability in cortisol responses. A number of features of the laboratory stress context were also manipulated with the intention of reducing habituation in cortisol response across repeated exposures (Study 3). The result was a more consistent elicitation of cortisol responses over repeated visits. This context was therefore considered suitable for the PL intervention (Study 4).

8.1.2 Cognitive Tests Sensitive to Stress

Tests of cognitive performance were administered under conditions of stress and non-stress in Studies 2 and 3. This was undertaken to identify specific tests sensitive to stress provocation to carry forward into the PL intervention study. Tests that engaged cognitive functions associated with the PFC were primarily assessed. Performance on two tests of WM and an executive control task-switch test were demonstrated to be sensitive to stress provocation to varying degrees, and in the presence and absence of a significant cortisol response. The observed performance impairment by stress exposure was considered sufficient to subsequently adopt these tests for administration in the PL intervention study.

8.1.3 Dispositional Factors, Cortisol Responsivity and Stress Vulnerability

The identification of stable dispositional predictors of cortisol responsivity was considered an important factor for the selection of a ‘stress vulnerable’ sample for the intervention study. The reasoning for this was two-fold. Firstly, impairment of cognitive performance is more consistently and significantly reported in individuals exhibiting pronounced cortisol responses to stress provocation. Secondly, the hypothesised protective effects of PL intake on stress responses, and therefore cognitive performance, may only be demonstrated by individuals characterised by some form of stress vulnerability. Therefore, stable trait personality factors specifically associated with cortisol responsivity were collected from participants across Studies 1 – 3 with the intention of identifying any dispositional factors associated with cortisol responsivity. A dimension of perfectionism associated with an increased need for precision, order, and control emerged as a consistent predictor of cortisol responses to acute stress across studies in this thesis; Perfectionism: Organisation was subsequently adopted as an inclusion criterion for the PL intervention study.

8.1.4 The Potential for Phospholipids to Moderate Stress and Protect Cognitive Performance

The laboratory stress context, cognitive tests sensitive to stress provocation, and perfectionist disposition associated with cortisol responsivity were employed in a randomised, placebo-controlled, double-blind study of the potential for 6 weeks PL intake to demonstrate protective effects on cognitive performance under conditions of acute stress (Study 4). Contrary to the hypothesised effects of attenuated stress responses and subsequent protective effects on cognitive performance, participants supplemented with PLs demonstrated increased cortisol, sympathetic and subjective arousal responses. Modest evidence of an attenuated anticipatory rise in subjective stress by PL intake was revealed. Definitive assessment of the potential for PLs to protect cognitive performance was undermined by variability in cognitive test performance aptitude between participants randomised to the two experimental conditions. However, no evidence of protective effects was revealed.

8.2 Discussion of Key Findings

8.2.1 Real-World or Laboratory Stress Context?

The decision to examine stress responses in the real-world or the laboratory may be considered a trade-off between naturalness and precision. Examination of the potential for assessing stress responses in a real-world context as part of this thesis was underpinned by a number of factors. Laboratory stress protocols typically employ artificial stimuli/tasks which are less likely to be intimately linked to genuine goals and identity. There is also some doubt regarding the validity of extrapolating findings from laboratory settings to responses elicited in the real world (Johnston et al., 2008; Lundberg et al., 1990; Van Doornen & Van Blokland, 1992; Van Eck et al., 1996). For example, laboratory stress paradigms frequently report lower cortisol responsivity in women and may under-estimate female cortisol response. (Kajantie & Phillips, 2006; Kudielka & Kirschbaum, 2005). Whilst sexual dimorphism in HPA axis-mediated stress responses is likely to be the primary factor, some of the variability in response may be explained by the types of stressors and the context employed. For example, men may exhibit heightened cortisol responses to competitive, achievement-based stressors, often employed in laboratory contexts, whilst women have been shown to demonstrate greater reactivity to interpersonal stressors (e.g., marital conflict; Kiecolt-Glaser et al., 1996; Stroud, Salovey,

& Epel, 2002). Interestingly, men and women tend to demonstrate comparable cortisol responses to exercise stressors further emphasising an important role for psychosocial factors in divergent cortisol responsivity (Friedmann & Kindermann, 1989; Kirschbaum, Wust, & Hellhammer, 1992; Kraemer, Blair, Kraemer, & Castracane, 1989). Women exhibited trends towards higher cortisol response to the naturalistic audition stressor (Studies 1 and 2), whilst men exhibited significant SBP responses in this context. This emphasises the importance of examining stress responsivity to different types of stressor both in the laboratory and natural settings to give further insight into sex-mediated variability in stress responses and the specific psychological factors that may underpin such differences.

8.2.1.1 The performance audition stress context

The evidence reported in this thesis demonstrated the performance audition context to be sufficient to provoke significant endocrine, physiological, psychological responses. The audition setting was also sufficiently structured to permit the collection of timely measures of physiological and subjective stress, and cognitive test performance. However, a lack of test-retest reliability in terms of cortisol response provocation was demonstrated. It was not possible to ascertain which specific psychological, physiological, or situational variables accounted for this lack of test-retest reliability. The auditions were comparable in terms of structure and timing and no significant differences in reported social-evaluative threat or subjective stress (peak) were observed. Due to the multiple and diverse influences on the HPA axis, studies, in which cortisol is a primary dependent variable, need to hold constant or control variables that affect HPA axis stress responses (e.g., physical and psychological health, smoking status, stage of menstruation, dietary status, and weight). Such control is often difficult to implement in real-world settings, wherein, variables are minimally controlled to retain the ecological validity of the setting. The capacity to provoke significant cortisol response has been demonstrated to vary *between* naturalistic stressors (Biondi and Picardi, 1999; Michaud, et al., 2008). Here, variability in the capacity for cortisol responses provocation was demonstrated *within* a naturalistic stress context. The reported test-retest reliability of standardised laboratory stressors often exceeds 70 - 80% (TSST; Kudielka, Hellhammer, & Wust, 2009). The variable capacity observed in the audition context may be explained by the uncontrolled and unstandardised nature of the real-world context; wherein extraneous variables influencing stress responsivity are both more likely and free to vary.

8.2.1.2 The laboratory stress context

The lack of reliability of the performance audition context led to the decision to pilot a laboratory stress protocol (Study 3). The increased control and standardisation afforded by this context permitted greater control over potential extraneous variables known to influence both cortisol responsivity, and importantly, cortisol response habituation (discussed further below). Subsequent manipulations of the study design to address such variables were made (see Section 6.1.2). The level of control and standardisation applied in the laboratory psychosocial stress protocol resulted in an overall consistency in response provocation within and between stress exposures.

An additional benefit of standardised laboratory stress protocols is their general suitability for use in diverse participants groups. Essentially, laboratory stressors can be administered to anyone recruited into a study. Protocols, such as the TSST, can be further adjusted to more adequately meet the needs and requirements of different groups (e.g., children [Buske-Kirschbaum et al., 1997]; groups [Childs, Vicini, & De Wit, 2006]). The audition stressor employed in Studies 1 and 2 permitted the examination of stress responses in a specific group of individuals who could be characterised by a number of dispositional and demographic factors specific to those that partake in competitive performance auditions. This can be considered a limitation of this type of naturalistic context; especially when compared to the capacity to recruit from the general population offered by the laboratory stressor employed. However, Study 1 demonstrated that the competitive performance arts context is a promising context in which to examine real-world stress responses. Involvement in a competitive performance activity offers an opportunity to examine stress responses in a context that involves a genuine personal goal and display of a valued skill, explicitly evaluated by others - central tenets of the Social Self-Preservation Theory (Dickerson, Gruenewald, & Kemeny, 2004; Dickerson & Kemeny, 2004). The audition stress context examined here may also be considered comparable to the TSST in form, structure, and psychological demand. Exposing auditionees to a TSST visit would provide an interesting comparison of stress reactivity both within individuals and across a comparable real-world and laboratory stress context.

8.2.1.3 The selection of a stress context

The trade-off between naturalness and precision when employing a real-world or laboratory stress context is illustrated by the studies presented in this thesis. The benefits of examining stress responses and cognitive performance under conditions of real-world

psychosocial stress can provide valuable insight into individual response and performance under stress in the real-world. This also permits verification of findings emerging from controlled laboratory studies. However, the lack of control and standardisation intrinsic to real-world contexts increases the risk of extraneous variables influencing the study outcomes and has the potential to reduce the reliability of significant responses being provoked. This is problematic considering the time and costs associated with undertaking experimental studies. Conversely, the increased control available in the laboratory reduces the influence of extraneous variables and increases test-retest reliability. This however, comes at the cost of naturalness as laboratory stress protocols may bear little resemblance to stress commonly experienced in the real-world. Both laboratory and naturalistic stressors have variable potencies in capacity to reliably provoke the HPA axis. Whilst examination of stress response and performance in natural, real-world settings should be strived for to assess the validity of extrapolating findings from laboratory contexts, the greater control and standardisation, and reliability in potency to provoke significant cortisol responses, ultimately led to the decision to employ a laboratory stress protocol in the PL intervention study (Study 4).

8.3 Habituation in Stress Responses to Repeated Stress Exposure

Within this thesis, habituation was considered as the reduction in magnitude and/or intensity of an initial psychoneuroendocrine stress response following repeated exposure to a homotypic stressor. The repeated measures design implemented in the PL intervention study required that the identified stress context should have the capacity to provoke significant and comparable cortisol responses over repeated exposures. Since the psychoneuroendocrine stress system is particularly prone to rapid response habituation (Engert et al., 2010; Federenko et al., 2004; Gerra et al., 2001; Jonsson et al.; Kirschbaum, Pruessner et al., 1995; Schommer et al., 2003; Wust et al., 2005a) it was important to demonstrate the reliability of a stress context to provoke repeated cortisol responses. Furthermore, the potential psychosocial factors which might influence habituation in response needed to be considered in the studies undertaken in this thesis to identify a suitable stress context for the PL intervention study. Hence, studies were designed to explore the feasibility of employing a real-world social-evaluative stressor, and manipulating contextual characteristics of a laboratory stressor, in an attempt to reduce habituation to confirm that this repeated measures design was suitable for the subsequent evaluation of the dietary intervention.

8.3.1 Habituation to Real-world Performance Stressors

In his seminal review, Mason (1968) proposed that habituation of HPA axis-mediated responses are likely to be moderated by situational factors such as novelty, predictability, controllability and anticipation of negative outcomes. Repeated exposure to a homotypic stressor is proposed to result in the reduction of perceived levels of novelty, unpredictability, and uncontrollability which will reduce the capacity of the stressor to provoke the HPA axis (Mason, 1968; Rose, 1984). Social-evaluative threat has since been proposed as an additional key psychological characteristic associated with activation of the HPA axis (Dickerson & Kemeny, 2004). Motivated performance related to an important goal, under conditions of social evaluation and uncontrollability, appears to be a key psychosocial determinant of cortisol responsivity to stress. Repeated exposures to laboratory stress characterised by social-evaluative threat often leads to habituation of cortisol response. This may be due to a combination of reduced novelty, unpredictability, uncontrollability, and a concomitant reduction in the potency of perceived social-evaluative threat. Evidence that the increased threat to social status, and personal significance attached to a real-world social-evaluative competitive performance stressor, may diminish habituation in cortisol response informed the decision to examine a similar stress context (Rohleder et al., 2007). However, this thesis does not add to the evidence in support of reduced habituation to real-world social-evaluative stressors. Difficulty ascertaining the number of auditions previously undertaken prevented analysis of this factor on cortisol responsivity (Study 1). The failure to elicit a cortisol response at an initial audition stress exposure (Study 2) resulted in the decision not to undertake a repeated audition stress exposure. The lack of significant cortisol response, despite no significant difference in the perceived level of social-evaluative threat between Studies 1 and 2, may in itself be considered incompatible with this hypothesis; or at least suggest that social-evaluative threat is not the only factor influencing the response.

8.3.2 Habituation to a Physical/Psychosocial Laboratory Stressor

The lack of reliability in the real-world context informed the decision to pilot a laboratory stress protocol (Study 3). Overall responses to the laboratory stressor were suggestive of comparable/sensitised response rather than significant habituation commonly reported in the majority of samples (e.g., Engert et al., 2010; Federenko et al., 2004; Gerra et al., 2001; Jonsson et al.; Kirschbaum, Pruessner, et al., 1995; Schommer et al., 2003; Wust et al., 2005a). Mean cortisol response to the psychosocial/physical laboratory stress protocol was characterised by heightened anticipatory pre-stress, and comparable peak

and post-stress responses at repeat stress exposure. This was demonstrated across stress visits in Study 3 and Study 4. Whilst the well-documented inter- and intra-individual variability in cortisol responsivity was evident within these samples, reflected in evidence of no cortisol response and the typical habituation in response in some individuals, the often observed overall tendency for habituated responses across the sample as a whole was not demonstrated. This suggests that there was less habituation in cortisol response in the laboratory stress context adopted here than in other psychosocial stress protocols reported in the literature. The increased level of control (e.g., dietary status and stressor onset) and standardisation (e.g., consistent method of stress induction) of the laboratory stressor are likely to have increased the reliability of this context to elicit cortisol stress responses. However, considering the tendency for habituation in other well-controlled, standardised laboratory stress protocols, this is not considered likely to have significantly contributed to the reduced habituation observed. Other potential factors that may have contributed to this effect are discussed below.

8.3.2.1 Combined physical/psychosocial stressor

Animal models suggest less rapid HPA axis response habituation to physical, rather than psychological stressors; particularly those involving a proximate physical threat (see Grissom & Bhatnagar, 2009). However, proximate physical threat in this context refers to serious threats to the organism's homeostasis (e.g., hypothermia and hypoglycaemia) rather than the mild physical pain elicited by the CPT (Emmert & Herman, 1999; Herman & Cullinan, 1997; Lovallo, 1975). Therefore, it is perhaps unlikely that the level of physical threat associated with the SECPT stressor employed in Studies 3 and 4 can fully account for the observed effects. It may be hypothesised that the psychological component of performing a physical task in a social-evaluative setting may amplify the stress-provoking nature of the stressor. The combination of a physical stressor and social-evaluation, rather than social-evaluation alone, has been demonstrated to be necessary for the SECPT to elicit a cortisol response (Schwabe & Wolf, 2010). This is suggestive of HPA axis activation under conditions in which individuals are concerned about self-presentation. Influenced by the Social Self-Preservation Theory, Rohleder et al. (2007) hypothesised that threats to the social self may, under certain circumstances, represent such a fundamental drive, akin to threats to the physical self, that cortisol responses may show reduced habituation (Rohleder et al., 2007). Whilst the threat to the social-self encountered in laboratory psychosocial contexts (e.g., TSST) may not be sufficient to sustain significant cortisol responses over repeated exposures in the majority, the addition of an evaluated physical stress component may act to increase the level of threat

experienced (particularly when evaluated by the opposite sex – discussed below). However, this hypothesised effect can only be speculated upon. Reliable activation of robust cortisol and BP responses by a combined social-evaluative and physical stress task has recently been reported. The Maastricht Acute Stress Test (MAST; Smeets et al., 2012) combines repeated social-evaluated CPTs and mental arithmetic. This stressor has been shown to elicit cortisol responses comparable to the TSST (Goff, Ali, & Pruessner, 2013; Smeets et al., 2012). However, to date there are no published reports of the capacity of this stressor, or the SECPT, to elicit cortisol responses over repeated exposures.

8.3.2.2 Manipulation of contextual variables

Situational variables such as novelty, uncontrollability, and unpredictability are proposed to be key determinants of HPA axis activation (Mason, 1968; Rose, 1984), and the reduced perception of these variables is associated with habituation in cortisol response to the same stressor (Harl et al., 2006; Schommer et al., 2003; Voigt et al., 1990). This generates the hypothesis that habituation to repeat exposure to a homotypic stressor may be reduced by manipulation of contextual and situational factors to increase the likelihood that the stressor is perceived as novel, unpredictable, and uncontrollable over repeated exposures. The contextual changes adopted across laboratory stress exposures may therefore have contributed to the observed cortisol response pattern. Despite contextual manipulations and minor changes to stressor tasks, the laboratory stress protocol was considered homotypic; that is, the changes employed did not result in the stress protocols being so different that they could be considered distinct stressors. For example, the speech task was different across the stress exposures. Schommer et al. (2003) propose that it is not the task per se that activates the HPA axis, rather the psychosocial social-evaluative context in which the task is performed. Support for this comes from evidence of activation of the HPA axis whilst performing a homotypic task (Stroop Test) only when completed under conditions of social-evaluation (Kirschbaum, Ebrecht, & Hellhammer, 2001). The stress induction room, panel, and experimenter were also changed across stress visits. However, the general structure and social-evaluative context was retained. Whilst manipulation of contextual and social-evaluative variables may have contributed to the capacity of the stress protocol to elicit significant cortisol responses across repeated exposures, similar manipulations have been employed for repeated administration of the TSST without significant attenuation of habituated response (e.g., Hellhammer et al., 2012; Schommer et al., 2003; Von Kanel et al., 2006). Therefore, the relative contribution

of attempts to retain novelty, unpredictability, and uncontrollability to habituation effects by manipulating situational/contextual factors may have been minimal.

8.3.2.3 Opposite sex effects

Evidence suggests that opposite sex effects (participants vs. stress panellists) are an important modulator in the elicitation of endocrine, sympathetic, and subjective stress responses to social-evaluative psychosocial stress (Larkin et al., 1998; Martinso & Zerface, 1970; Roney et al., 2007; Roney & Simmons, 2008; Roney et al., 2010). This effect appears relevant to both men and women (Goff et al., 2013). The relative contribution of this factor to the observed cortisol responses over repeated exposures cannot be elucidated without a same sex social-evaluative panel comparison condition. Furthermore, there are no published studies which have specifically examined opposite sex social-evaluative stress over repeated exposures. However, considering evidence of the propensity of social-evaluation from the opposite sex to amplify cortisol stress responses (e.g., Duchesne et al., 2012) it is plausible that the addition of an opposite sex social-evaluative panel contributed to ensuring the stressor retained a sufficient level of social-evaluative threat over repeated exposures.

8.3.2.4 Frequency of stress exposure

The frequency of exposure to a homotypic stressor has been associated with cortisol response habituation in animals (Grissom & Bhatnagar, 2009). However, evidence of reduced habituation over longer inter-stressor delays in humans has received modest support (Petrowski et al., 2012). Therefore, the only consistent evidence of the effects of stressor interval on reduced habituation comes from animal data (De boer et al., 1990; Desouza & Van Loon, 1982; Ma & Lightman, 1998). The potential influence of the temporal interval between exposures on the laboratory stressor employed in this thesis is difficult to ascertain without data on repeated responses over varying time intervals. Manipulation of time intervals between stressor exposures would be needed to characterise the contribution of temporal effects. However, it seems unlikely that temporal delay would be the only contributing factor, given the evidence of habituation to psychosocial stress exposure over longer delays than the six weeks employed here (e.g., Hellhammer et al., 2012).

8.3.3 Anticipatory Effects of Repeated Stress Exposure

Exposure to the laboratory stressor was characterised by pronounced anticipatory cortisol responses at the second exposure. The increased anticipatory response to repeated exposure to the laboratory stressor was mirrored in HR and subjective stress responses but not BP response (in the absence of PL intake). Heightened cortisol responsivity in the anticipation of stress has previously been demonstrated in men (Kirschbaum, Wust, & Hellhammer, 1992). Trends towards this response have also been reported in repeated laboratory psychosocial stress studies (e.g., Kirschbaum et al., 1995); although not consistently (e.g., Gerra et al., 2001; Petrowski et al., 2012). However, the anticipatory cortisol response elicited by the laboratory stress protocol administered in this thesis was notable for its significant magnitude. This could reflect heightened subjective stress sensitivity; likely underpinned by the negative expectancy of the type of stressor to be faced. However, despite a concurrent elevation in anticipatory subjective stress, very little association was revealed between anticipatory cortisol and subjective stress responses. The significant elevation may be explained by the physical nature of the stress protocol. Physically challenging stressors have previously been associated with increased anticipatory cortisol response (Mason et al., 1973; Salvador, Suay, Gonzalez-Bono et al., 2003; Sutton & Casey, 1975). Moreover, the heightened repeated stress anticipatory response was most evident in those demonstrating habituated or sensitised responses to repeated stress exposure (Study 3). This suggests that anticipatory responses are associated with particular responder 'types'. Engert et al. (2013) have recently proposed a differentiation between anticipatory and reactive cortisol responder types. Anticipatory responders demonstrate significantly elevated cortisol responses 10-16 minutes after the onset of anticipation and are characterised by heightened peak cortisol response. Reactive cortisol responders do not demonstrate significant elevations in cortisol until approximately 10 minutes after the onset of stress. This distinction between reactive and anticipatory responder types has yet to be examined in relation to cortisol responses over repeated stress exposures. However, it may be hypothesised that individuals characterised by heightened anticipatory cortisol responses may be more likely to demonstrate sensitised cortisol response profiles. However, contrary to this hypothesis cortisol habituators also demonstrated heightened anticipatory responses (Study 3), and evidence has demonstrated that heightened anticipatory responses are associated with blunted cortisol response profiles (exam stress; Young & Nolen-Hoeksema, 2001). Here (Study 3), the small numbers of participants categorised as habituated and sensitisated was too small (n = 4) to allow any meaningful statistical analysis of this potential

relationship. Further research into the distinction between anticipatory and reactive responder types to single and repeated acute stress exposure is warranted considering evidence of anticipatory responses being specifically associated with a number of negative life events (e.g., early life adversity [Hardie, Moss, Vanyukov, Yao, & Kirillovac, 2002]; PTSD [Bremner et al., 2003]; and phobia [Alpers, Abelson, Wilhelm, & Roth, 2003]).

8.3.4 Differentation of Habituation in Response Systems

Evidence provided throughout this thesis offers some support for the hypothesis that habituation patterns of stress responses are variable depending upon the specific response system. For example, sympathetic responses to stress are often considered to be more uniform in activation (Schommer et al., 2003). Indeed, in the absence of PL intake, BP responses over repeated laboratory stress exposures were shown to be analogous. Heart rate and subjective stress responses were however characterised by a shift in response kinetic towards heightened anticipatory responses. The collection of a mid-stress measure of subjective stress (Study 4) further revealed this increased anticipatory response during repeated stress exposure to be accompanied by an attenuation of a response peak initially demonstrated during stress.

8.3.5 Summary

Taken together, the research presented in this thesis has revealed that the combination of a physical and psychosocial stress context reduced the tendency towards the well-documented habituation in cortisol response. The specific situational or psychosocial factors underpinning this effect have been explored and are worthy of further investigation. Considering previous evidence of cortisol habituation to psychosocial stressors which manipulate contextual factors (e.g., stress room and panel), and the mixed human evidence of the effects of inter-stressor delay, these findings suggest that the addition of a socially-evaluated physical stressor and/or opposite sex evaluation may be prime candidates underlying the observed effects. However, based on the available evidence, this hypothesis is speculative. Replication of this effect in larger sample sizes is also required. With regards to the aims and objectives of this thesis, the overall tendency towards comparable cortisol responses over repeated exposures further supported the decision to employ the laboratory stressor in the PL intervention study.

8.4 Sensitivity of Cognitive Test Performance to Stress Impairment

Findings presented in this thesis support the hypothesis that PFC-mediated cognitive function is vulnerable to the impairing effects of stress. In accordance with the literature, this relationship was not always straight-forward and was moderated by a number of factors. Working memory has been demonstrated to be vulnerable to the stress exposure in real-world (e.g., Klein & Boals, 2001b; Robinson et al., 2008) and laboratory (e.g., Elzinga & Roelofs, 2005; Schoofs et al., 2008; Luethi et al., 2008) contexts. Corroborative impairment of WM was observed both in the absence of a cortisol response (Study 2), and in the presence of a response (moreover, amplified in those exhibiting the highest responses [Study 3]) of cortisol. This is compatible with the animal (de Kloet et al., 1999) and human depletion-replacement (Lupien et al., 2002) evidence whereby GCs moderate cognitive performance in an inverted-U function; i.e., performance is impaired by excessively low or high GC levels. Conversely, cortisol may be sufficient, but not necessary, for the moderation of performance. Whilst cortisol is commonly emphasised as the key moderating factor in the effects of stress on memory, adrenergic arousal has also been shown to affect the relationship; both in isolation (e.g., Birnbaum et al., 2000; Robbins, 2005; Usher et al., 1999) and in synergy with cortisol (e.g., Elzinga & Roelofs). Furthermore, other cognitive factors are likely to contribute to observed effects. Performance on WM tasks requires focussed attention to be paid to task-relevant demands, and off-task demands to be ignored or suppressed (Klein & Boals, 2001b). Attentional resources are limited (Hiller, Futner, Sachse, & Martini, 2012). Therefore, cognitive interference in the form of unwanted or distracting thoughts, or the mental effort required to suppress such thoughts, can function as an impairing factor by reducing resources available for proficient cognitive performance (Stawski et al., 2009). However, little support was revealed for the contributory effects of cognitive interference (both general and related to stress exposure) in this thesis. Furthermore, the primary role for cortisol was demonstrated in the impairment of WM performance only reaching significance (2-back), and increased significance of impairment (A-ospan), in those exhibiting the highest cortisol responses (Study3).

The sensitivity of the WM tests utilised in the thesis demonstrate the fact that cognitive tests engaging multiple WM processes are more vulnerable to the impairing effects of stress (Schoofs et al., 2008; Schoofs et al., 2009). Some of the reported inconsistency in the impairing effects of stress on WM may be interpreted in relation to the level of demand placed on cognitive resources. For example, studies employing WM memory

tests which require only passive maintenance and repetition of information (e.g., digit span forward) often report no effect of stress (Hoffman & Al'Absi, 2004; Kuhlmann et al., 2005; Smeets et al., 2006). Furthermore, performance impairment on the digit span backward (repetition of a series of digits in reverse order), which places more cognitive demand on WM resources, has been demonstrated to be impaired by psychosocial stress whilst no effects on the digit span forward were revealed (Schoof & Wolf, 2009). Thus, less demanding tasks are not sensitive enough to index small changes in performance; especially those induced by experimental manipulations in healthy, young individuals as examined across this thesis (Reynolds, 1997; Unsworth & Engle, 2005).

Emerging evidence of tasks of executive control being sensitive to the effects of stress (Plessow et al., 2012; Steinhauser et al., 2007) was supported by the studies conducted in this thesis. Specifically, task-switching performance was demonstrated to be impaired under conditions of stress. This impairment was specific to task-switch performance accuracy (Study 3), whereas, RT performance was unaffected by stress exposure (replicating Plessow et al., 2012). The specificity of the effect is not surprising given that RT and accuracy measures in task-switching tests may not reflect the same underlying mechanisms (Altmann & Gray, 2008). Such findings suggest reduced proficiency of executive control under conditions of stress. Whilst this domain of cognitive performance was impaired by stress exposure, this effect was not augmented in high cortisol responders as was demonstrated for tests of WM. This may reflect divergent underlying mechanisms, or primacy of cortisol as a moderating factor, in the impairment of these PFC-mediated functions. Indeed, the current stress and executive control literature emphasises cognitive resource allocation competition mechanisms as responsible for the impairing effect of stress on this cognitive domain (Plessow, Fischer, Kirschbaum, & Goschke, 2011). Although no significant effects of cognitive distraction/interference were demonstrated in the studies presented in this thesis, it may be that more sophisticated measures are needed to examine potential cognitive load factors which contribute to the observed impairment of executive control performance.

The examination of PFC-mediated cognitive performance under conditions of stress in this thesis has revealed a number of findings that contribute to the field of stress and cognition, for instance, demonstration of impaired performance on two WM tests that engage multiple, and divergent, WM processes. Furthermore, the demonstration of this impairment in the absence of cortisol, and dependent upon cortisol (impairment in high cortisol responders), suggests that the underlying mechanisms contributing to these effects require elucidation. The evidence of the impairment of executive control processes

adds important information to this research area and, coupled with the lack of impairment observed in high cortisol responders, raises questions regarding the factors which modulate of this cognitive function during/following stress. With regard to the aims and objectives of the thesis, the piloting of cognitive tests prior to undertaking the planned PL intervention study was considered an important prerequisite to examining the potential for PL intake to offer protective effects on performance. This was considered essential to ensure any potential effects of PL intake on performance were not inhibited by a lack of sensitivity of cognitive tests employed. The importance of examining the sensitivity of cognitive tests to the effects of stress was emphasised by the lack of significant effects of stress on the VISGED test. Evidence of impaired performance on the n-back, A-ospan, and task-switching test were considered sufficient to adopt these measures for the PL intervention study.

8.5 Predictors of Cortisol Responsivity

8.5.1 Perfectionism and Stress Responsivity

Response specificity refers to the concept that particular characteristics of a stimulus or individual are associated with distinct neuroendocrine or physiological responses (Bosch et al., 2009). The specific characteristics of stress contexts associated with activation of the HPA axis have been reported (Dickerson & Kemeny, 2004; Mason, 1968; Rose, 1984). Less consistent evidence of an association of specific individual characteristics with cortisol responsivity has been demonstrated (Blood et al., 1994; Kirschbaum, Bartussek et al., 1992; Salmon et al., 1989; Schommer et al., 1999; Schoofs et al., 2008a; Van Eck et al., 1996). The collection of trait personality data in this thesis identified perfectionism as a dispositional characteristic associated with acute cortisol responsivity. Perfectionism had predictive validity for cortisol responses both in a naturalistic and a laboratory social-evaluative context. Predictive validity appears to be associated with higher responsivity but not higher responses that fail to habituate. There has only been one previous study that has reported the predictive capacity of this personality trait in relation to cortisol response to acute stress (Wirtz et al., 2007).

The multidimensional nature of perfectionism has been emphasised and reflected in the instruments developed to measure this disposition (Frost et al., 1990; Hewitt & Flett, 1991; Slaney, Rice, Mobley, Trippi, & Ashby, 2001). Two multidimensional perfectionism scales have predominated in the research literature: the Frost (1990; employed in this

thesis), and Hewitt and Flett's (1991) Multidimensional Perfectionism Scales. Both scales underscore the multifaceted nature of perfectionism. The Frost et al. scale emphasises the setting of excessive standards, self-critical evaluation, and doubts about actions. This scale also incorporates parental influences (parental criticism and expectations) and a need for order and organisation. Hewitt and Flett's (1991) scale extends the dimensions of perfectionism into the interpersonal realm comprising self-orientated perfectionism (excessive personal standards and evaluation of own behaviour), socially-prescribed perfectionism (ideation that others hold excessively high expectations and punitive judgement of the respondent), and other-orientated perfectionism (excessive expectation of others).

Perfectionism has been clearly associated with increased psychological distress, including depression (Blatt, 1995; Hewitt & Flett, 1991; Shafran & Mansell, 2001), hopelessness (O'Connor & O'Connor, 2003), and suicidality (Hunter & O'Connor, 2003). Such associations with negative psychological states suggest a role for perfectionism as a stress-vulnerability factor within a diathesis-stress paradigm (Chang, Watkins, & Banks, 2004; O'Connor & O'Connor, 2003). In general, the assumption is that several facets of perfectionism serve as stress-vulnerability factors and moderate stress reactions. For example, the relationship between perfectionism and stress may be moderated by an enhanced appraisal of events as stressful (Hewitt et al., 2002; Hewitt & Flett, 1993; Hewitt, Flett, & Ediger, 1996). The cognitive style associated with perfectionism may increase the tendency to magnify the negative aspects of stressful experiences. Moreover, a perfectionist cognitive style may not only result in heightened appraisal of stress, but in more frequent generation of stressful experiences. Excessively high standards, self-criticism, and doubts about performance likely increase the likelihood that high perfectionists will find performance tasks and contexts, particularly ones that are socially-evaluated as employed in this thesis, more threatening (Flett et al., 1991; Shafran & Mansell, 2001). Perfectionists are also more likely to derive their sense of self-worth from performance level so the potential for failure, or to be judged as less than perfect, is likely to be particularly stressful (Hewitt & Flett, 1993). Ego-involvement is emphasised as a key psychological component of the Social Self-Preservation Theory (Dickerson & Kemeny, 2002, 2004; Dickerson, Mycek, & Zaldivar, 2008). Therefore, greater importance attached to performance, and a combination of excessively high standards and self-worth arising from level of performance, may result in perfectionists being more vulnerable to stress; particularly social-evaluative threat. Empirical support for a link between

perfectionist characteristics and psychological stress has been reported (Dunkley, Zuroff, & Blankstein, 2003; Tashman, Tenenbaum, & Eklund, 2012).

8.5.2 Perfectionism: Organisation and Stress Responsivity

Considering the evidence that perfectionism is a stress-vulnerability factor for psychological distress it is not surprising that a relationship between cortisol response and perfectionism was revealed in the studies presented in this thesis. However, the specific dimension of perfectionism that emerged as important was surprising. Perfectionism: Organisation – the exacting over-emphasis of precision, order and organisation – is considered the least interlinked dimension of perfectionism in Frost et al's (1990) scale. Perfectionism is hypothesised by some to contain both adaptive and maladaptive characteristics (Bieling, Israeli, Smith, & Antony, 2003; Cox, Enns, & Clara, 2002; Enns, Cox, Sareen, & Freeman, 2001; Slade & Owens, 1998). Indeed, Frost et al. (1993) considered Perfectionism: Personal Standards and Organisation to be 'positive striving' characteristics of perfectionism. Therefore, Perfectionism: Organisation may be considered a positive form of functioning associated with setting high personal standards and striving for excellence associated with positive outcomes (Bieling, Israeli, & Antony, 2004; Brown et al., 1999; Flett et al., 1995), whilst maladaptive forms (e.g., Concern over Mistakes, Doubts about Actions) are associated with negative outcomes (e.g., higher perceived stress; Chang et al., 2004). Indeed, Wirtz et al. (2007) reported a relationship between such maladaptive dimensions and cortisol responsivity. However, the distinction between adaptive and maladaptive forms of perfectionism is not always consistently observed. Both adaptive and maladaptive perfectionism are associated with eating disorders (Bardone-Cone et al., 2007). Moreover, adaptive forms of perfectionism have also been associated with negative psychological distress (Bieling et al., 2004; Cox et al., 2002; Hill, McIntire, & Bacharach, 1997), and higher levels of perceived hassles (Dunkley, Blankstein, Halsall, Williams, & Winkworth, 2000). Thus, a distinction between adaptive and maladaptive forms of perfectionism may underlie positive and negative outcomes respectively under normal conditions. However, under conditions of stress, perfectionist tendencies may, in general, become vulnerability factors.

The need for order, precision, and organisation may be threatened under uncontrollable social-evaluative stress conditions. Therefore, those individuals with a high need for order, control, and organisation may present with heightened cortisol responsivity. Some support for this hypothesis may be provided by evidence of heightened cortisol responses to a social-evaluative stressor in high conscientiousness individuals (Garcia-Banda et al.,

2011). Conscientiousness is associated with efficiency, organisation, neatness, and need for achievement (Costa & McCrae, 1992) and has been shown to significantly correlate with Perfectionism: Organisation (Cox et al., 2002). Taken together, this evidence suggests heightened need for order and organisation may be associated with increased cortisol responsivity to psychosocial stress. However, perfectionism and conscientiousness are characterised by distinct elements of behavioural disposition.

8.5.3 Potential Factors Mediating the Relationship between Perfectionism and Stress

The relationship between perfectionism and psychological stress is not consistently reported (Rice & Van Arsdale, 2010). There is also evidence of a negative relationship between aspects of perfectionism and stress suggesting increased stress-coping capacity in perfectionists (Stoll, Lau, & Stoeber, 2008). Inconsistency in research findings may highlight a potential role for factors mediating the relationship between stress and perfectionism. Coping style (Dunkley et al., 2000; Dunn, Whelton, & Sharpe, 2006), self-esteem (Rice, Ashby, & Slaney, 1998), and ruminative thought (O'Connor et al., 2007) have been demonstrated to mediate the relationship between perfectionist traits and psychological distress. For example, rumination – broadly characterised by repetitive, unwanted, past-orientated thoughts of negative content (Zoccola & Dickerson, 2012) – has been demonstrated to be a key mediating factor in the maladaptive effects of perfectionism. O'Connor and O'Connor (2003) and Flett et al. (2002) have demonstrated that ruminative brooding – a passive comparison of present condition with an unachieved standard (Treynor, Gonzalez, & Nolen-Hoeksema, 2003) – is an important mechanism that mediates the maladaptive effects of perfectionism (particularly socially prescribed perfectionism) on psychological distress. Therefore, whilst Perfectionism: Organisation emerged as a significant predictor of cortisol responsivity, this relationship may be further mediated by additional factors not measured in this thesis. The relationship between perfectionism and cortisol responsivity, and potential mediating factors, is worthy of further examination.

8.5.4 Association between Dispositional Predictors and Stress over Repeated Stress Exposures

Stable predictors of cortisol response may only emerge once the potential masking influence of initial stressor novelty has receded. Indeed, stressor novelty has been identified as an important factor in elicitation of stress responses (Epstein, 1986; Epstein

& Obrien, 1985; Voigt et al., 1990). Moreover, trait personality factors related to response disposition may only emerge over repeated stress exposures (Kirschbaum, Bartussek et al., 1992; Pruessner, Gaab et al., 1997). The lack of significant relationship between Perfectionism: Organisation and cortisol responses at a repeat stress exposure (Study 3) would seem to support this and suggest this dimension may be specifically associated with higher cortisol responses in situations that are novel. However, high perfectionists exhibited comparable cortisol responses at the repeat stress visit suggesting that a response sensitisation in low perfectionist participants appears the more likely cause of loss of predictive power. Further evidence of the importance of assessing cortisol responses over repeated exposures comes from the emergence of alexithymia EOT as a predictor of anticipatory cortisol response at the repeat stress exposure, and a relationship between net cortisol response and self-esteem once cortisol responses were aggregated. Interestingly, genetic heritabilities in cortisol stress responses may also only emerge once the initial novelty, and heightened anxiety, associated with initial exposure to a stressor are reduced following repeat exposure (Federenko et al., 2004).

8.5.5 Summary

Considering the potentially numerous factors mediating the relationship between disposition and stress response, and the emergence of moderating personality characteristics over the changing situational context of repeated stress exposure, the association between cortisol responsivity and character is too complex and multifaceted to be fully elucidated from evidence generated by studies presented in this thesis. However, evidence for a relationship between perfectionism and cortisol responsivity can contribute to current understanding and aid future examination of cortisol response specificity. In terms of the aims of this thesis, the positive association between perfectionism and cortisol responsivity was considered sufficient to adopt this disposition to identify those likely to demonstrate heightened cortisol responsivity for the PL intervention study.

8.6 Phospholipids, Stress and Cognitive Performance

Overall, the findings from the PL intervention study (Study 4) can be seen to add to the existing heterogeneous evidence of the potential for dietary PLs to moderate stress and cognitive performance. Endocrine, physiological, and subjective responses revealed contrasting effects of PL supplementation. The effect of PL intake on cortisol response was inconsistent with the hypothesised stress attenuating capacity of these lipids. This

contrasts with evidence of the cortisol attenuating effects of PS (Fahey & Pearl, 1998; Monteleone et al., 1990; Monteleone et al., 1992; Starks et al., 2008) and a PL complex (Hellhammer et al., 2004). However, the lack of significant overall effect of PL intake, and trends for increased cortisol output, is consistent with other reported effects (Hellhammer et al., 2012; Hellhammer et al., 2010; Parker et al., 2011; Schubert et al., 2011). Differences in the types/sources of PL administered and the nature of the stressor eliciting the cortisol response may underpin some of the heterogeneity. The most consistent cortisol attenuating effects of (bovine cortex and soy-derived) PL have been reported in recovery from exhaustive physical exercise in males. This may indicate a specifity of cortisol attenuating effect, in predominantly well-trained individuals, post-intensive exercise. Hellhammer et al. (2004) reported the only clear-cut attenuation of cortisol responsivity by soy-derived PL intake in a psychosocial stress context. Studies administering bovine milk-derived PLs have since failed to replicate such findings. The attenuation of cortisol response in a post-hoc sub-sample of high stress-load participants (Hellhammer et al., 2010) suggests the stress-buffering effects of such PLs may be specific to chronically stressed individuals. However, subsequent studies recruiting high stress-load individuals, whilst highlighting the importance of controlling for stress-load, did not demonstrate attenuation in cortisol response (Hellhammer et al., 2012; Schubert et al., 2010). Indeed, these studies reported trends for increased (acute and CAR) responses evident here.

Considering the hypothesised inverted U-shaped relationship between cortisol and cognitive performance (de Kloet et al., 1999), individuals that present with stress responses attenuated by high chronic stress-loads may be vulnerable to deleterious effects of an under-responsive stress system; including impaired cognitive performance (Lupien, McEwen, Gunnar, & Heim, 2009). This raises the hypothesis that PL intake may have potential protective effects in high stress-load individuals via the augmentation of cortisol response to functional levels. This thesis focussed upon individuals that may be characterised by a tendency for high cortisol responsivity rather than those exhibiting high chronic stress-loads that may be more prone to demonstrate attenuated responses. This decision was predominantly based upon the stress and cognition literature. It was considered that more foundation could be given to the hypothesis that reducing the cortisol response had the potential to protect cognitive performance than the hypothesis that increasing cortisol response in low responders would restore cortisol to functional levels. Furthermore, as previously noted, evidence for the moderation of cortisol response by PLs in high stress-load individuals is mixed. Due to evidence of an association

between chronic stress and attenuated cortisol responsivity, individuals reporting high chronic stress-load were excluded from studies across this thesis and chronic stress-load of eligible participants was included in statistical models. Whilst chronic stress rating did not significantly contribute to observed cortisol responses in the PL intervention study, it is noted that the sample selected for this study reported chronic stress scores on the margins of the cut-off scores used by Hellhammer and colleagues (2010, 2012) to categorise high and low stress-load participants. Therefore, more stringent stress-load control measures may have proved beneficial. Overall, evidence from this thesis suggests that PL intake does not significantly attenuate cortisol responses in young healthy males. Indeed, a trend for increased response was evident. Further research might therefore, be best focussed upon identifying specific sub-populations in which the modulatory effects of PLs previously reported may be more consistently demonstrated.

Phospholipid intake instigated increased physiological and subjective indices of sympathetic arousal. The reported effects of augmented BP were unexpected and are unprecedented in the PL and stress literature. The mechanisms underlying this effect can only be speculated upon due to a lack of previous evidence of the modulation of sympathetic response by PLs. Whilst the published data do not report any previous evidence supporting the potential for PL intake to moderate sympathetic reactivity to acute stress, considering the high probability that PL intervention studies are likely to often be industry funded, there may exist a bias towards non-publication of negative results. However, this can only be speculated upon. Moreover, this bias will likely be offset by the listing of studies on clinical trials registers. Potential mechanisms of action include the fatty acid composition of the PLs administered and the permissive effects of cortisol on the sympathetic actions of catecholamines. Further examination of the parameters of acute sympathetic stress responses would be needed to clarify if the specific plasma fatty acid composition (treatment effect) or co-occurrence of cortisol and catecholamines (integrated stress response effect) underlies this relationship.

Evidence of heightened subjective arousal in the PL condition substantiates the effect of PLs on sympathetic tone. Further support for this being an effect of PL intake comes from evidence of habituation in subjective arousal to the laboratory stress context both in the control condition and in responses demonstrated in Study 3. In contrast, PL intake marginally significantly attenuated an increased anticipatory subjective stress response. Whilst a marginal effect, this adds to previous evidence of the psychological stress-buffering effects of PLs (Benton et al., 2001; Fahey & Pearl, 1998; Hellhammer et al., 2004; Hellhammer et al., 2010). The rating of subjective stress on the SACL corresponds

to feelings of pleasantness and unpleasantness (Mackay, 1978). This subjective rating of stress relates to the appraisal of a stimulus as challenging or threatening (Ursin & Eriksen, 2004). The arousal dimension of the SACL relates to the perceived level of autonomic arousal. This differentiation in subjective response may reflect the capacity of PL intake to increase autonomic response, whilst attenuating perception of threatening aspects of the stressor. A BM-PL drink has previously been demonstrated to reduce perceived stress and increase perceived controllability (Hellhammer et al., 2010). The effects of PL on subjective stress observed here were only demonstrated in anticipation of stress exposure. This may be suggestive of an anticipatory subjective stress-reducing effect of PL intake. However, this assertion is contradicted by an association between subjective stress and SBP. Therefore, the reduced subjective stress rating may be more likely reflective of a potential anxiolytic effect of heightened cortisol at the corresponding measurement time point (0 minutes; Serkan Het & Wolf, 2007; Schlotz et al., 2008).

Unfortunately, conclusions on the protective effects of PL-intake on cognitive performance under conditions of stress are tempered by the underlying differences in cognitive test aptitude evident between participants randomised to the drink conditions. Controlling for the divergent baseline performance revealed no significant, consequential, effects of PL intake. Naturally, such findings are treated with caution due to the failure of randomisation to adequately control divergent test aptitude and the potential for reduced opportunity for improvement in PL participants due to ceiling effects, or potential regression to the mean in this condition. However, this evidence contributes to the lack of current substantial support for the protective effects of PL intake on cognitive performance under stress.

8.7 Limitations of the Thesis Methodologies

A number of limitations of the methods employed across the thesis are acknowledged.

8.7.1 Compliance

As with any dietary intervention studies carried out in a free-living context, full compliance with study protocol cannot be assured. Compliance with study drink intake in Study 4 was self-reported since drinks were consumed each morning at home. Whilst drink intake diaries, face-to-face compliance meetings, and the prospect of salivary ‘compliance checks’ may have increased the likelihood of compliance, differences in frequency of drink intake across conditions cannot be ruled out.

8.7.2 Test Drink Composition

Many of the previous studies reporting protective effects of PL intake administered PS whilst a PL compound drink, of which PS was a relatively minor component, was administered here. However, the amount of PS (300 mg/d) was comparable to previous studies. The administration of a compound drink means the relative contribution of specific PLs to the observed effects cannot be established from the evidence presented here. This would have limited the precision of any conclusions should any protective effects have been revealed. Specific PLs included in the PL-rich drink may have potential protective effects on cognition and stress response. For example, sphingomyelin and phosphatidylcholine were the main PLs in the test drink. Sphingomyelin is found in high quantities in the brain and neural tissues (Ohlsson, Burling, Duan, & Nilsson, 2010). Phosphatidylcholine is the major dietary source of choline and plays a vital role in neuronal membranes. Dietary augmentation of this compound increases the availability of the precursors of acetylcholine synthesis and may subsequently increase availability of this key memory-related neurotransmitter (McDaniel et al., 2003). However, animal in vitro bioavailability evidence (Wurtman et al., 2006) and supplementation studies of human memory (Becker & Giacobini, 1988; Growdon, Corkin, Huff, & Rosen, 1986) provides little support for this effect. Future attempts to characterise the potential stress-buffering effects of PL intake may benefit from the administration of specific PL lipids in isolation. Furthermore, considering evidence of variability in the effects of PL intake on stress response as a function of dose (Hellhammer et al., 2004), the administration of variable doses of individual PLs would give a better indication of potential functional benefits of PL intake.

8.7.3 Speculative Mechanisms of Action

Proposed mechanisms of PLs action after supplementation are underpinned by the assumption that exogenously administered PLs can reach the inner leaflet of cell membranes to exert biochemical action. However, the mechanism via which PLs are incorporated into cell membranes, and the specific effects upon cell membrane composition, are poorly characterised in humans. Furthermore, the mechanisms by which PLs may exert hypothesised effects on HPA axis-mediated stress responses are particularly poorly explicated and can be considered speculative at best. More clearly defined mechanisms for hypothesised actions of PLs on psychoneuroendocrine function are required to better understand why these lipids have the capacity to attenuate and augment cortisol responses to stress. More clearly characterised underlying mechanisms

may also give a better understanding of the selectivity and inconsistency of the observed effects. Furthermore, further clarification is needed with regards the intake period necessary to sufficiently alter brain levels of PL and the bioavailability of these lipids during dietary intake.

8.7.4 Randomisation Issues and Matching of Intervention Conditions

The issue of divergent cognitive test aptitude undermining the examination of the potential protective effects of PL intake can be considered a difficult effect to control. Random allocation to drink conditions should reduce the likelihood that characteristics and aptitudes of individuals in each condition will be relatively comparable. This likelihood of this effect will naturally diminish if larger samples are recruited. However, an alternative approach would be to assign participants to conditions in a quasi-random manner based upon established baseline test aptitude. Alternative study designs may also have reduced the randomisation issues observed. For example, a crossover design would have removed the potential for divergent cognitive test aptitude across condition as a function of individual differences.

8.7.5 Other Factors Moderating Impairing Effects of Stress on Cognition

The focus upon the moderating effects of cortisol on impairment of cognitive function was reflective of evidence in the literature and in acknowledgement of the potential mechanism of PL intake attenuating the cortisol response thus proffering protective potential on function. However, it is unlikely that cortisol alone moderates cognitive function under stress. Indeed, findings from the studies in this thesis lend support to this supposition. Further, the role of CRH, vasopressin and ACTH as well as adrenergic arousal and cognitive factors have all been demonstrated to play a role in the effects of stress on cognitive function (De Vito et al., 2009; Roozendaal, Brunson, Holloway, McGaugh, & Baram, 2002; Stawski et al., 2009). It is likely that endocrine, sympathetic, and cognitive factors interact on numerous levels. Measures collected could have been more reflective of this interaction. For example, more detailed measures of cognitive distraction/interference would have given a clearer indication of the potential role of psychological/cognitive factors in the moderation of cognition by stress. However, the collection of multiple measures during completion of cognitive tasks was avoided due to the danger of overloading participants which may have affected performance in itself. It is

acknowledged that the collection of state ruminative/preservative thought measures post-stress exposure would have given valuable insight as such cognitive factors have been associated with both increased and prolonged cortisol responsivity (Zoccola, Quas, & Yim, 2010), and cognitive impairment (Stawski et al., 2009). However, the time needed to complete such measures and potential extra cognitive load on participants precluded the collection of such measures. Similarly, measures of coping styles may have provided further insight into the relationship between stress responsivity and disposition. Whilst some credence was given to this potential mediating factor in the use cognitive appraisal scales (PASA and PSRS), specific measures of coping style may have been beneficial to further explore the variability in stress responses.

8.7.6 Effects of Sex on Generalisability to other Samples

The decision to recruit a male only sample naturally diminished the generalisability of the findings from Studies 3 and 4. Studies examining acute cortisol responses often exclude female participants from studies owing to the sex dimorphism in HPA axis-mediated stress responses. Therefore, this control measure is commonly adopted in the studies of stress and cognition (Domes, Heinrichs, Rimmele, Reichwald, & Hautzinger, 2004; Kuhlmann et al., 2005; Luethi et al., 2009; Schoofs et al., 2009; Smeets et al., 2006; Takahashi et al., 2004; Tollenaar et al., 2009) and stress and PL intake (Baumeister et al., 2008; Benton et al., 2001; Fahey & Pearl, 1998; Hellhammer et al., 2012; Hellhammer, Waladkhani, Hero, & Buss, 2010; Monteleone et al., 1990; Monteleone et al., 1992; Parker et al., 2011; Schubert et al., 2011; Starks et al., 2008). However, it would have been advantageous to explore the thesis aims of Studies 3 and 4 in both males and females. Particularly considering previous evidence of the effects of PL intake are almost exclusively confined to male samples. It would also be of interest to examine the effect of exposure to the laboratory stressor employed here in female participants.

8.7.7 Subjective Measurement Issues

Measurement of levels of perceived social-evaluative threat during laboratory studies would have permitted comparisons of the importance of this factor relative to elicited responses to naturalistic and laboratory stressors. Whilst characteristics of the laboratory stressor employed have previously been shown to be high in social-evaluative threat (Dickerson & Kemeny, 2004), the use of more comprehensive measures of social evaluative threat or measures of shame and negative self-evaluation (see Gruenewald et al., 2004) would have also given a more detailed insight into the relationship between

specific aspects of social-evaluative threat and cortisol response. Furthermore, the potential contribution of variables such as perceived controllability, novelty, and predictability to observed habituation patterns should have been assessed to better identify factors underlying the cortisol responsivity over repeated stress exposure.

Studies across this thesis cast some doubt upon the suitability of the POMS (McNair, 1971) and the POMS-SF (Shacham, 1983) for assessing subjective transient mood states in the stress contexts employed. Results demonstrated mixed results from this measure. Significant mood disturbances were revealed using the full length POMS in Study 1. However, the time to complete this measure and the reported effect on fatigue resulted in the use of the short-form in Studies 2 and 3. Mixed results were suggestive of variability in the sensitivity of the POMS-SF to assess mood changes. Finally, the full version was employed at less frequent intervals to that employed in Study 1 for the PL intervention study. Whilst significant mood changes were revealed it would be preferable to record more frequent subjective mood ratings across the response profile. Indeed, the collection of regular subjective measures across the full temporal response profile has recently been highlighted (Hellhammer & Schubert, 2012) and was supported by evidence of a peak subjective stress response mid stressor (Study 4). Therefore, future studies may benefit from alternative, shorter measures to assess subjective mood at regular intervals.

8.7.8 Relationship between Dispositional Characteristics and Cortisol Responsivity

The FMPS (Frost et al., 1990) was adopted due to the previous reported association between dimensions of this scale and cortisol response (Wirtz et al., 2007). However, it is acknowledged that other scales of multidimensional perfectionism may have offered further insight into the relationship between this trait and responsivity. For example, Hewitt and Flett's (1991) Multidimensional Perfectionism Scale has been more widely employed and closely associated with psychological stress responses. The additional employment of this scale would have given more scope for comparing the relationship between perfectionism and cortisol observed in this thesis and the perfectionism and psychological distress literature. However, it is noted that the Hewitt and Flett scale does not include a dimension directly comparable to Perfectionism: Organisation so the observed relationship may not have been revealed. The FMPS has also received criticism related to the number of factors underlying the scale (Stober, 1998). It is also acknowledged that the trait variables collected across the thesis to explore the relationship between disposition and cortisol responsivity was not exhaustive. Additional

characteristics have been associated with cortisol responses to stress. For example, Chida & Hamer, 2008 reported that positive psychological states (e.g., positive affect) are associated with reduced HPA axis stress reactivity. As previously discussed, rumination has been demonstrated to be associated with basal and reactive cortisol response, and to play a mediatory role between perfectionism and psychological stress. Trait rumination has been associated with cortisol responsivity and recovery, although this relationship is inconsistently reported and is likely underpinned by the extent to which depression is represented in the rumination scale employed (Zoccola & Dickerson, 2012). Therefore, it is acknowledged that the collection of measures of ruminative tendency may have given further insight into the dispositional character associated with increased cortisol responsivity.

The relationship between pre- and early postnatal stress and HPA axis responsivity in later life has received increasing support (Huizink, Mulder, & Buitelaar, 2004; Weinstock, 2005; Weinstock, 2008; Wust, Entringer, Federenko, Schlotz, & Hellhammer, 2005). The 'fetal origin hypothesis' proposes that maternal prenatal stress (as well as malnutrition, smoking, drug use, and preeclampsia; Kramer, 2003) results in adverse outcomes for the fetus such as low birth weight and increased vulnerability to negative health outcomes in later life (Barker, 1999, 2002; Barker, Osmond, Rodin, Fall, & Winter, 1995). Low birth weight has been shown to be inversely related to salivary cortisol response to acute psychosocial stress in adult and juvenile males (Jones et al., 2006; Wust, Entringer, Federenko, Schlotz, & Hellhammer, 2005). This relationship has also been demonstrated in following pharmacological stimulation (dexamethasone suppression test; Kajantie et al., 2003). Further evidence suggests early atypical or stressful rearing experience may also predict cortisol responsivity to psychosocial stress in later life. Examples include childhood abuse or maltreatment (Heim, Mletzko, Purselle, Musselman, & Nemeroff, 2008; Heim, Newport, Bonsall, Miller, & Nemeroff, 2001) and experience of parental death (Tyrka et al., 2008). It is therefore acknowledged that the collection of measures of pre- and early postnatal stress would have given further insight into characteristics and factors moderating HPA axis reactivity in studies conducted across this thesis.

8.8 Future Directions

This thesis examined the effects of a PL intervention in a young healthy sample. The potential protective effects of PL intake may be limited to individuals who are perhaps more likely to be compromised cognitively than young high functioning students. The observed modulatory effects of dietary interventions on cognitive performance are often

small and inconsistently reported (McDaniel et al., 2003). Hence, the sensitivity of such manipulations may only be significantly demonstrated in sub-groups with specific nutritional or cognitive vulnerabilities. For example, the impairing effect of breakfast omission on cognitive performance in children is often only shown in under- and malnourished children (Chandler, Walker, Connolly, & Grantham-mcgregor, 1995; Pollitt, Cueto, & Jacoby, 1998; Pollitt, Jacoby, & Cueto, 1996; Pollitt, Leibel, & Greenfield, 1981) or children with lower IQ (Pollitt & Mathews, 1998). Protective effects of dietary interventions on cognitive performance may also be limited to those that are cognitively or metabolically at risk, such as the elderly (Manning, Ragozzino, & Gold, 1993; Messier, Gagnon, & Knott, 1997; Parsons & Gold, 1992). For example, glucose administration has been shown to selectively enhance cognitive performance in elderly, but not young participants (Hall, Gonderfrederick, Chewning, Silveira, & Gold, 1989; Manning, Parsons, Cotter, & Gold, 1997). Indeed, observed protective effects of PL intake on visual memory under conditions of stress have been reported only in a post-hoc categorised older sub-group (Schubert et al., 2011). Therefore, it is proposed that future studies examining the effects of PL intake on cognitive performance under stress should focus upon specific population subgroups perhaps more likely to benefit from any protective effects of PL supplementation.

The primary aims and objectives of the thesis prevented further examination of the psychological and contextual variables that may underpin the variability of the audition stress context to consistently perturb the HPA axis. Similarly, the possibility that naturalistic stress contexts may be sufficient to reduce the tendency of habituated cortisol responses over repeated exposures is worthy of future examination. Of particular interest is the variability in stress responsivity between men and women under different contextual and psychological stressor conditions. Whilst only trends of higher cortisol responses to the audition stress exposure in women were observed, this is in direct contrast to the commonly demonstrated heightened responsivity in men in laboratory psychosocial stress contexts. Considering the similarities between the audition stress context and laboratory stress protocols, such as the TSST, further exploration of stress responses in men and women across both contexts may provide interesting insights into potential variables underlying response specificity.

Further examination of cortisol responses to the laboratory stress protocol described herein may be warranted. Particular attention should be paid to identifying the relative importance of potential contributory factors to cortisol responses over repeated exposures (e.g., socially-evaluated physical stressor, opposite sex evaluation, inter-stressor delays

etc.). This would require manipulations of each aspect to isolate significantly contributory factors to maintenance/sensitisation of salivary cortisol response. It is also necessary to examine if the reduced overall habituation can be replicated and if administration of the SECPT alone (removal of the TSST speech element) is sufficient to provoke comparable responses over repeated stress exposures. The observed reduced habituation may not be solely representative of the stress context employed, rather a reflection of the inter-individual differences in cortisol responsivity to repeated stress. Whilst overall habituation in cortisol response patterns to repeated laboratory psychosocial stressors is typically demonstrated, this response pattern does not necessarily occur in all individuals. Aside from emphasising situational factors that moderate the activation of HPA axis stress responses, Mason (1968) also emphasised that the HPA axis is characterised by large inter-individual differences. Wust et al. (2005), despite reporting habituation in cortisol responses in the sample as a whole, demonstrated that considerable inter-individual variability in responses also held true for responses to repeated stress. A substantial number of males (male twin pairs [monozygotic and dizygotic]) demonstrated reduced or sensitised habituation to repeated TSST exposure. Therefore, the laboratory stress context described in this thesis needs to be examined in larger samples to rule out the possibility that inter-individual differences in habituation, rather than specific situational characteristic of the manipulated stress context, underlie the overall reduced response habituation.

Examination of the potential for stress to impair WM performance to date has often failed to adequately distinguish which specific WM processes are affected. Whilst evidence in this thesis supports the hypothesis that WM memory tests that engage multiple and complex WM processes may be sensitive to impairment (Elzinga & Roelofs, 2005; Schoofs et al., 2008; Schoofs et al., 2009), insight into which specific processes are impacted upon by stress provocation remains unclarified. Further examination delineating which WM processes are explicitly impaired by stress would elucidate the relationship between stress and this cognitive function further. A number of questions also remain with regards to the time-course effects of acute stress on PFC-mediated cognitive functions. For example, impairment of task-switching performance has been demonstrated both immediately after stress provocation and over 40 minutes after stress onset (in this thesis and Plessow et al., 2011). Conversely, WM has been shown to be characterised by a variable time course of impairment. This includes impairment only during concurrent cortisol and SNS activation; impairing effects were abolished as SNS activation subsided as the time period after stressor exposure increased (Elzinga &

Roelofs, 2006). Schoofs et al. (2008) demonstrated impaired n-back performance only in the first two of eight stimulus trial blocks (10 minutes post-stress). This may be suggestive of a temporal effect of stress on the modulation of WM that is not evident for impaired task-switching performance. Further examination is required to accumulate empirical evidence to define the nature of the relationship between stress and executive control processes. For example, it is not known whether this relationship is linear or inverted U-shaped in nature (Plessow et al., 2011).

8.9 Conclusions

Taken together, the studies presented in this thesis demonstrate the ecological validity of a performance audition context in the elicitation of significant stress responses. However, inconsistency in the provocation of cortisol responses in this context highlighted the potential for reduced test-retest reliability in naturalistic settings. The methodological modification of the TSST was sufficient to reduce the level of cortisol habituation inherent in repeated testing. The additions of a physical stressor and an opposite sex social-evaluative panel are potential moderators of this effect. Prefrontal cortex-mediated cognition was demonstrated to be sensitive to stress, but an inconsistent role of cortisol in this effect was observed. Working memory and executive control were demonstrated to be sensitive to the impairing effects of stress in the real-world and laboratory. The role of cortisol in this impairment varied as a function of cognitive test and magnitude of cortisol response. Working memory was impaired in the presence and absence of cortisol and in high responders. Executive control was impaired in the presence of cortisol but not as a function of cortisol response magnitude. This suggests additional factors, or more likely, multiple factors operating on a number of levels, contribute to the impairment of these cognitive functions by stress. Chronic phospholipid intake was associated with an increase in cortisol, cardiovascular and subjective arousal responses following acute stress provocation. A modest attenuation of anticipatory subjective stress was observed but overall the stress-buffering potential of phospholipids was not supported. Future examination of this potential in cognitively vulnerable groups is recommended. Finally, this thesis also identified a dispositional characteristic associated with cortisol responsivity to acute stress. The confirmation of Perfectionism: Organisation as a predictor of cortisol responsivity is an important finding worthy of examination to further elucidate the relationship between this personality trait and stress responsivity.

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Appendix 1 Salivary Cortisol Collection Standard Operating Procedure

SOP for CORTISOL COLLECTION

Intended use:

To obtain measures of salivary cortisol.

Description of use:

Saliva collection will be performed no earlier than 1 hour after a meal, smoking, drinking caffeine/acidic drinks or brushing teeth in order to ensure there is no contamination of the saliva with interfering substances.

The swab will be removed from the suspended insert by the participant and gently chewed for 1 minute to produce a sufficient quantity of saliva. In case of doubt, the participant will be asked to keep the swab in his/her mouth until he/she feels he/she can no longer prevent himself/herself from swallowing the saliva produced.

Afterwards the participant will replace the swab in the suspended insert (plastic tube where the swab is stored in the salivette tubing) and the salivette will be firmly closed using a stopper which clips over the insert and outer casing.

All samples will be frozen by the researcher in the Institute of Psychological Sciences Human Appetite and Research Unit until analysis. The samples will be appropriately transported to Arla Foods research laboratory in Stockholm for cortisol assay analysis. Samples will be centrifuged for a minimum of 2 minutes at 1,000 x g to yield a clear saliva sample for analysis. Cortisol assay analysis will be undertaken using a Salimetrics salivary cortisol enzyme immunoassay kit.

Important Notes:

As a matter of principle, the minimum volume recovered should be 1ml of saliva.

Supplies:

Sarstedt salivettes

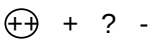
Maintenance Plan:

The expiration date of all salivettes will be checked before testing. Any material out of date will be disposed of. Samples will be sent for analysis to Arla Foods research facility in Stockholm and subsequently destroyed. Samples are likely to be analysed before 2011.

Appendix 2 The Stress and Arousal Checklist

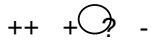
The adjectives shown below describe different feelings and moods. Please use this list to describe your feelings at this moment in time.

If the adjective definitely describes your feelings circle the:

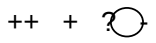


If the adjective more or less describes your feelings circle the:

If you do not understand the adjective, or you cannot decide whether it describes how you feel circle the:



If the adjective does not describe the way you feel circle the:



Your first reactions will be the most reliable, therefore do not spend too long thinking about each adjective. Please be as honest and accurate as possible.

Tense	++ + ? -	Tired	++ + ? -
Relaxed	++ + ? -	Idle	++ + ? -
Restful	++ + ? -	Up tight	++ + ? -
Active	++ + ? -	Alert	++ + ? -
Apprehensive	++ + ? -	Lively	++ + ? -
Worried	++ + ? -	Cheerful	++ + ? -
Energetic	++ + ? -	Contented	++ + ? -
Drowsy	++ + ? -	Jittery	++ + ? -
Bothered	++ + ? -	Sluggish	++ + ? -
Uneasy	++ + ? -	Pleasant	++ + ? -
Dejected	++ + ? -	Sleepy	++ + ? -
Nervous	++ + ? -	Comfortable	++ + ? -
Distressed	++ + ? -	Calm	++ + ? -
Vigorous	++ + ? -	Stimulated	++ + ? -
Peaceful	++ + ? -	Activated	++ + ? -

Appendix 3 The Profile of Moods States

INSTRUCTIONS:

Below is a list of words that describe feelings people have. Please read each one carefully. Then circle the number to the right which best describes how you are feeling **at this moment in time**. The numbers correspond to the descriptions at the top of each column (0 = "Not at all", 4 = "Extremely").

		Not at all	A little	Moderately	Quite a bit	Extremely
1	**Friendly**	**0**	**1**	**2**	**3**	**4**
2	**Tense**	**0**	**1**	**2**	**3**	**4**
3	**Angry**	**0**	**1**	**2**	**3**	**4**
4	**Worn out**	**0**	**1**	**2**	**3**	**4**
5	**Unhappy**	**0**	**1**	**2**	**3**	**4**
6	**Clear-headed**	**0**	**1**	**2**	**3**	**4**
7	**Lively**	**0**	**1**	**2**	**3**	**4**
8	**Confused**	**0**	**1**	**2**	**3**	**4**
9	**Sorry for things done**	**0**	**1**	**2**	**3**	**4**
10	**Shaky**	**0**	**1**	**2**	**3**	**4**
11	**Listless**	**0**	**1**	**2**	**3**	**4**
12	**Peeved**	**0**	**1**	**2**	**3**	**4**
13	**Considerate**	**0**	**1**	**2**	**3**	**4**
14	**Sad**	**0**	**1**	**2**	**3**	**4**
15	**Active**	**0**	**1**	**2**	**3**	**4**
16	**On edge**	**0**	**1**	**2**	**3**	**4**
17	**Grouchy**	**0**	**1**	**2**	**3**	**4**
18	**Blue**	**0**	**1**	**2**	**3**	**4**
19	**Energetic**	**0**	**1**	**2**	**3**	**4**
20	**Panicky**	**0**	**1**	**2**	**3**	**4**
21	**Hopeless**	**0**	**1**	**2**	**3**	**4**
22	**Relaxed**	**0**	**1**	**2**	**3**	**4**
23	**Unworthy**	**0**	**1**	**2**	**3**	**4**
24	**Spiteful**	**0**	**1**	**2**	**3**	**4**
25	**Sympathetic**	**0**	**1**	**2**	**3**	**4**
26	**Uneasy**	**0**	**1**	**2**	**3**	**4**
27	**Restless**	**0**	**1**	**2**	**3**	**4**
28	**Unable to concentrate**	**0**	**1**	**2**	**3**	**4**
29	**Fatigued**	**0**	**1**	**2**	**3**	**4**
30	**Helpful**	**0**	**1**	**2**	**3**	**4**
31	**Annoyed**	**0**	**1**	**2**	**3**	**4**
32	**Discouraged**	**0**	**1**	**2**	**3**	**4**
33	**Resentful**	**0**	**1**	**2**	**3**	**4**
34	**Nervous**	**0**	**1**	**2**	**3**	**4**

		Not at all	A little	Moderately	Quite a bit	Extremely
35	**Lonely**	0	1	2	3	4
36	**Miserable**	0	1	2	3	4
37	**Muddled**	0	1	2	3	4
38	**Cheerful**	0	1	2	3	4
39	**Bitter**	0	1	2	3	4
40	**Exhausted**	0	1	2	3	4
41	**Anxious**	0	1	2	3	4
42	**Ready to fight**	0	1	2	3	4
43	**Good natured**	0	1	2	3	4
44	**Gloomy**	0	1	2	3	4
45	**Desperate**	0	1	2	3	4
46	**Sluggish**	0	1	2	3	4
47	**Rebellious**	0	1	2	3	4
48	**Helpless**	0	1	2	3	4
49	**Weary**	0	1	2	3	4
50	**Bewildered**	0	1	2	3	4
51	**Alert**	0	1	2	3	4
52	**Deceived**	0	1	2	3	4
53	**Furious**	0	1	2	3	4
54	**Efficient**	0	1	2	3	4
55	**Trusting**	0	1	2	3	4
56	**Full of pep**	0	1	2	3	4
57	**Bad-tempered**	0	1	2	3	4
58	**Worthless**	0	1	2	3	4
59	**Forgetful**	0	1	2	3	4
60	**Carefree**	0	1	2	3	4
61	**Terrified**	0	1	2	3	4
62	**Guilty**	0	1	2	3	4
63	**Vigorous**	0	1	2	3	4
64	**Uncertain about things**	0	1	2	3	4
65	**Bushed**	0	1	2	3	4

Appendix 4 The Profile of Mood States – Short Form

INSTRUCTIONS:

Please circle the number which best describes how you are feeling **at this moment in time**. The numbers correspond to the descriptions at the top of each column (0 = "Not at all", 4 = "Extremely").

		Not at all	A little	Moderately	Quite a bit	Extremely
1	Tense	0	1	2	3	4
2	Peeved	0	1	2	3	4
3	Sad	0	1	2	3	4
4	Hopeless	0	1	2	3	4
5	Restless	0	1	2	3	4
6	Active	0	1	2	3	4
7	Bewildered	0	1	2	3	4
8	Discouraged	0	1	2	3	4
9	Fatigued	0	1	2	3	4
10	Anxious	0	1	2	3	4
11	Cheerful	0	1	2	3	4
12	Uncertain about things	0	1	2	3	4
13	Exhausted	0	1	2	3	4
14	Blue	0	1	2	3	4
15	Miserable	0	1	2	3	4
16	Angry	0	1	2	3	4
17	Worthless	0	1	2	3	4
18	Annoyed	0	1	2	3	4

		Not at all	A little	Moderately	Quite a bit	Extremely
19	Vigorous	0	1	2	3	4
20	Grouchy	0	1	2	3	4
21	Resentful	0	1	2	3	4
22	On edge	0	1	2	3	4
23	Bitter	0	1	2	3	4
24	Unable to concentrate	0	1	2	3	4
25	Furious	0	1	2	3	4
26	Full of pep	0	1	2	3	4
27	Uneasy	0	1	2	3	4
28	Lively	0	1	2	3	4
29	Nervous	0	1	2	3	4
30	Bushed	0	1	2	3	4
31	Helpless	0	1	2	3	4
32	Confused	0	1	2	3	4
33	Unhappy	0	1	2	3	4
34	Energetic	0	1	2	3	4
35	Forgetful	0	1	2	3	4
36	Worn out	0	1	2	3	4
37	Weary	0	1	2	3	4

Appendix 5 Primary Appraisal Secondary Appraisal

PASA
Primary Appraisal Secondary Appraisal Scale

Name…….
Date…….

The following sentences refer to the oncoming situation. Please indicate what goes through your mind regarding all these sentences by ticking the respective circle. For each sentences, you can thereby indicate how much you agree or disagree with the statement. Please answer all sentences by circling the appropriate number.

	Totally disagree	Rather disagree	Disagree to some extent	Agree to some extent	Rather agree	Totally agree
I do **not** feel threatened by the situation	□	□	□	□	□	□
The situation is important to me.	□	□	□	□	□	□
In this situation I know what I can do.	□	□	□	□	□	□
It mainly depends on me whether the experts judge me positively.	□	□	□	□	□	□
I find this situation very unpleasant.	□	□	□	□	□	□
I do not care about this situation.	□	□	□	□	□	□
I have **no idea** what I should do now.	□	□	□	□	□	□
I can best protect myself against failure in this interview through my behavior.	□	□	□	□	□	□
I **do not** feel worried because the situation does not represent any threat for me.	□	□	□	□	□	□
The situation is **not** a challenge for me.	□	□	□	□	□	□
In this situation I can think of lots of action alternatives.	□	□	□	□	□	□
I am able to determine a great deal of what happens in this interview myself.	□	□	□	□	□	□
This situation scares me.	□	□	□	□	□	□
This task challenges me.	□	□	□	□	□	□
I can think of lots of solutions for solving this task.	□	□	□	□	□	□

Appendix 6 Perceived Stress Reactivity Scale

Perceived Stress Reactivity Scale

1 When tasks and duties build up to the extent that they are hard to manage.....
- ☐ I am generally untroubled
- ☐ I usually feel a little uneasy
- ☐ I normally get quite nervous

2 When I want to relax after a hard day at work.....
- ☐ This is usually quite difficult for me
- ☐ I usually succeed
- ☐ I generally have no problem at all

3 When I have conflicts with others that may not be immediately resolved.....
- ☐ I generally shrug it off
- ☐ It usually affects me a little
- ☐ it usually affects me a lot

4 When I make a mistake.....
- ☐ In general, I remain confident
- ☐ I sometimes feel unsure about my abilities
- ☐ I often have doubts about my abilities

5 When I am wrongly criticised by others.....
- ☐ I am normally annoyed for a long time
- ☐ I am annoyed just for a short time
- ☐ In general, I am hardly annoyed at all

6 When I argue with other people....
- ☐ I usually calm down quickly
- ☐ I usually stay upset for some time
- ☐ It usually takes me a long time until I calm down

7 When I have little time for a job to be done...
- ☐ I usually stay calm
- ☐ I usually feel uneasy
- ☐ I usually get quite agitated

8 When I make a mistake....
- ☐ I am normally annoyed for a long time
- ☐ I am normally annoyed for a while
- ☐ I generally get over it easily

9 When I am unsure what to do or say in a social situation....
- ☐ I generally stay cool
- ☐ I often feel warm
- ☐ I often begin to sweat

10 When I have spare time after working hard....
- ☐ It is often difficult for me to unwind and relax
- ☐ I usually need some time to unwind properly
- ☐ I am usually able to unwind effectively and forget about the problems of the day

11 When I am criticised by others....
- ☐ Important arguements usually come to my mind when it is too late to still make my point
- ☐ I often have difficulty finding a good reply
- ☐ I usually think of a reply to defend myself

Continued on next page...

12 When something does not go the way I expected.....
- [ ] I usually stay calm
- [ ] I often get uneasy
- [ ] I usually get very agitated

13 When I do not attain a goal....
- [ ] I usually remain annoyed for a long time
- [ ] I am usually disappointed, but recover soon
- [ ] In general, I am hardly concerned at all

14 When others criticise me....
- [ ] I generally don't lose confidence at all
- [ ] I generally lose a little confidence
- [ ] I generally feel very unconfident

15 When I fail at something.....
- [ ] I usually find it hard to accept
- [ ] I usually accept it to some degree
- [ ] In general, I hardly think about it

16 When there are too many demands on me at the same time.....
- [ ] I generally stay calm and do one thing after the other
- [ ] I usually get uneasy
- [ ] Usually, even minor interruptions irritate me

17 When others say something incorrect about me...
- [ ] I usually get quite upset
- [ ] I normally get a little bit upset
- [ ] In general, I shrug it off

18 When I fail at a task.....
- [ ] I usually feel very uncomfortable
- [ ] I usually feel somewhat uncomfortable
- [ ] I n general, I dont mind

19 When I argue with others...
- [ ] I usually get very upset
- [ ] I usually get a little bit upset
- [ ] I usually don't get upset

20 When I am under stress....
- [ ] I usually can't enjoy my leisure time at all
- [ ] I usually have difficulty enjoying my leisure time
- [ ] I usually enjoy my leisure time

21 When tasks and duties accumulate to the extent that they are hard to cope with.....
- [ ] My sleep is unaffected
- [ ] my sleep is slightly disturbed
- [ ] my sleep is very disturbed

22 When I have to speak in front of other people...
- [ ] I often get very nervous
- [ ] I often get somewhat nervous
- [ ] In general, I stay calm

23 When I have many tasks and duties to fulfill....
- [ ] In general, I stay calm
- [ ] I usually get impatient
- [ ] I often get irritable

Appendix 7 Perceived Stress Scale

Perceived Stress Scale

1. In the last month, how often have you been upset about something that has happened unexpectedly?	0	1	2	3	4
2. In the last month, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4
3. In the last month, how often have you felt nervous and "stressed"?	0	1	2	3	4
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	0	1	2	3	4
5. In the last month, how often have you felt things were going your way?	0	1	2	3	4
6. In the last month, how often have you found that you could not cope with all the things that you have had to do?	0	1	2	3	4
7. In the last month, how often have you been able to control the irritations in your life?	0	1	2	3	4
8. In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
9. In the last month, how often have you been angered because of things that were outside your control?	0	1	2	3	4
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

Appendix 8 Hospital Anxiety and Depression Scale

1. I feel tense or 'wound up':
- Most of the time ☐
- A lot of the time ☐
- From time to time, occasionally ☐
- Not at all ☐

2. I still enjoy the things I used to enjoy:
- Definitely as much ☐
- Not quite so much ☐
- Only a little ☐
- Hardly at all ☐

3. I get a sort of frightened feeling as if something awful is about to happen:
- Very definitely and quite badly ☐
- Yes, but not too badly ☐
- A little, but it doesn't worry me ☐
- Not at all ☐

4. I can laugh and see the funny side of things:
- As much as I always could ☐
- Not quite so much now ☐
- Definitely not so much now ☐
- Not at all ☐

5. Worrying thoughts go through my mind:
- A great deal of the time ☐
- A lot of the time ☐
- From time to time, but not too often ☐
- Only occasionally ☐

6. I feel cheerful:
- Not at all ☐
- Not often ☐
- Sometimes ☐
- Most of the time ☐

7. I can sit at ease and feel relaxed:
- Definitely ☐
- Usually ☐
- Not Often ☐
- Not at all ☐

8. I feel as if I am slowed down:
- Nearly all the time ☐
- Very often ☐
- Sometimes ☐
- Not at all ☐

9. I get a sort of frightened feeling like 'butterflies' in the stomach:
- Not at all ☐
- Occasionally ☐
- Quite Often ☐
- Very Often ☐

10. I have lost interest in my appearance:
- Definitely ☐
- I don't take as much care as I should ☐
- I may not take quite as much care ☐
- I take just as much care as ever ☐

11. I feel restless as I have to be on the move:
- Very much indeed ☐
- Quite a lot ☐
- Not very much ☐
- Not at all ☐

12. I look forward with enjoyment to things:
- As much as I ever did ☐
- Rather less than I used to ☐
- Definitely less than I used to ☐
- Hardly at all ☐

13. I get sudden feelings of panic:
- Very often indeed ☐
- Quite often ☐
- Not very often ☐
- Not at all ☐

14. I can enjoy a good book or radio or TV program:
- Often ☐
- Sometimes ☐
- Not often ☐
- Very seldom ☐

Appendix 9 Health Screening Questionnaire

PARTICIPANT SCREENING QUESTIONNAIRE

Thank you for agreeing to take part in this study. The following questionnaire is designed to collect information about your health and well being in order to assess your suitability for participation in the study.

You are under ***no obligation*** to answer any question that you may not wish to answer for any reason. Your confidentiality will be respected at ALL times.

Date of contact _____ /_____ /_____ Researcher…….

How did you find out about the study? Contacted by us ☐

Poster advert ☐

Word of Mouth ☐

Other ☐

CONTACT INFORMATION

Name……..

Address……

……

……

Contact phone number……

E-mail ……

Date of Birth ____ / ____ /____ Age …….

Measured height…… Measured weight……..

Measured BMI ……..kg/m²

HEALTH

How would you rate your general health……

Do you have or have you had any medical conditions? (e.g. asthma, diabetes) ……

……

Do you currently or have you previously experienced any heart complaints, chest pain or high blood pressure?

Yes ☐

No ☐

Resting BP ..... (160/95 mmHg?)

Do you experience any skin complaints on your hands/arms?

Yes ☐

No ☐

Do you experience any circulatory problems (e.g. reduced blood flow to finger or toes)?

Yes ☐

No ☐

Current medications ……

Do you smoke? No, never smoked ☐

Yes ☐

Given up ☐ How long ago?.....

Have you taken any recreational drugs in the last month

Yes ☐

No ☐

Do you currently work night shifts -

Yes ☐

No ☐

Food Preferences

During the study you will be provided with a test lunch on each of the three session visits. The meal provided will be a risotto. You will also be provided with water. Please indicate you food preference choices below.

Are there any foods you will not eat?

Details .....

Do you suffer from any food allergies (e.g. nut allergy)?

Yes ☐ Details .....

No ☐

Do you have any dietary requirements?

Yes ☐ Details .....

No ☐

OTHER INFORMATION

Can we keep this information on file and contact you about future studies? Yes / No

Signed ..... Date _____ /_____ /_____

Appendix 10 Frost Multidimensional Perfectionism Scale

FROST MULTIDIMENSIONAL PERFECTIONISM SCALE

Date: ..... Participant ID: _ _ _ _ _ _ _ _

Please read the following statements carefully and indicate the extent to which you agree each statement describes **you .** Please answer as honestly as you can. Your responses will be treated as confidential. Circle only one answer for each question.

	Strongly Agree	Agree	Neither Agree or Disagree	Disagree	Strongly Disagree
1. My parents set very high standards for me.	1	2	3	4	5
2. Organization is very important to me.	1	2	3	4	5
3. As a child, I was punished for doing things less than perfectly.	1	2	3	4	5
4. If I do not set the highest standards for myself, I am likely to end up a second-rate person.	1	2	3	4	5
5. My parents never tried to understand my mistakes.	1	2	3	4	5
6. It is important to me that I be thoroughly competent in everything I do.	1	2	3	4	5
7. I am a neat person.	1	2	3	4	5
8. I try to be an organized person.	1	2	3	4	5
9. If I fail at work/school, I am a failure as a person.	1	2	3	4	5
10. I should be upset if I make a mistake	1	2	3	4	5
11. My parents wanted me to be the best at everything.	1	2	3	4	5
12. I set higher goals for myself than most people.	1	2	3	4	5
13. If someone does a task at work/school better than me, then I feel like I failed the whole task.	1	2	3	4	5
14. If I fail partly, it is as bad as being a complete failure.	1	2	3	4	5
15. Only outstanding performance is good enough in my family.	1	2	3	4	5
16. I am very good at focusing my efforts on attaining a goal.	1	2	3	4	5
17. Even when I do something very carefully, I often feel that it is not quite done right.	1	2	3	4	5
18. I hate being less than the best at things.	1	2	3	4	5
19. I have extremely high goals.	1	2	3	4	5
20. My parents have expected excellence from me.	1	2	3	4	5

	Strongly Agree	Agree	Neither Agree or Disagree	Disagree	Strongly Disagree
21. People will probably think less of me if I make a mistake.	1	2	3	4	5
22. I never felt like I could meet my parents' expectations.	1	2	3	4	5
23. If I do not do as well as other people, it means I am an inferior human being.	1	2	3	4	5
24. Other people seem to accept lower standards from themselves than I do.	1	2	3	4	5
25. If I do not do well all the time, people will not respect me.	1	2	3	4	5
26. My parents have always had higher expectations for my future than I have.	1	2	3	4	5
27. I try to be a neat person.	1	2	3	4	5
28. I usually have doubts about the simple everyday things I do.	1	2	3	4	5
29. Neatness is very important to me.	1	2	3	4	5
30. I expect higher performance in my daily tasks than most people.	1	2	3	4	5
31. I am an organized person.	1	2	3	4	5
32. I tend to get behind in my work because I repeat things over and over.	1	2	3	4	5
33. It takes me a long time to do something 'right'.	1	2	3	4	5
34. The fewer mistakes I make, the more people will like me.	1	2	3	4	5
35. I never felt like I could meet my parents' standards	1	2	3	4	5

Appendix 11 Rotter's Locus of Control Scale

LOCUS OF CONTROL SCALE

Date: ..... Participant ID: _ _ _ _ _ _ _ _

Please read the following statement pairs carefully. For each of the 13 questions, tick the box next to **ONE** of the statements that best describes how your opinion.

1	Many of the unhappy things in people's lives are partly due to bad luck	☐
	People's misfortunes result from the mistakes they make.	☐
2	One of the major reasons why we have wars is because people don't take enough interest in politics.	☐
	There will always be wars, no matter how hard people try to prevent them.	☐
3	In the long run, people get the respect they deserve in this world.	☐
	Unfortunately, an individual's worth often passes unrecognized no matter how hard he tries.	☐
4	The idea that teachers are unfair to students is nonsense.	☐
	Most students don't realize the extent to which their grades are influenced by accidental happenings.	☐
5	Without the right breaks, one cannot be an effective leader.	☐
	Capable people who fail to became leaders have not taken advantage of their opportunities.	☐
6	No matter how hard you try, some people just don't like you.	☐
	People who can't get others to like them don't understand how to get along with others.	☐
7	I have often found that what is going to happen will happen.	☐
	Trusting to fate has never turned out as well for me as making a decision to take a definite course of action.	☐
8	In the case of the well prepared student, there is rarely, if ever, such a thing as an unfair test.	☐
	Many times exam questions tend to be so unrelated to course work that studying is really useless.	☐
9	Becoming a success is a matter of hard work; luck has little or nothing to do with it.	☐
	Getting a good job depends mainly on being in the right place at the right time.	☐

Please continue on next page

10	The average citizen can have an influence in government decisions.	☐
	This world is run by the few people in power, and there is not much the little guy can do about it.	☐
11	When I make plans, I am almost certain that I can make them work.	☐
	It is not always wise to plan too far ahead because many things turn out to be a matter of luck anyway.	☐
12	In my case, getting what I want has little or nothing to do with luck.	☐
	Many times we might just as well decide what to do by flipping a coin.	☐
13	What happens to me is my own doing.	☐
	Sometimes I feel that I don't have enough control over the direction my life is taking.	☐

Appendix 12 Rosenberg Self-Esteem Scale

ROSENBERG SELF-ESTEEM SCALE

Date: ..... Participant ID: _ _ _ _ _ _ _ _

The following questionnaires relate to how you feel about **yourself**. Please indicate the extent to which you agree with the following statements from strongly agree to strongly disagree. Please answer as truthfully as you can. Your responses will be treated as confidential. Circle only one answer for each question

	Strongly agree	Agree	Disagree	Strongly disagree
On the whole I am satisfied with myself	1	2	3	4
At times I think I am no good at all	1	2	3	4
I feel that I have a number of good qualities	1	2	3	4
I am able to do things as well as most people	1	2	3	4
I feel I do not have much to be proud of	1	2	3	4
I certainly feel useless at times	1	2	3	4
I feel that I am a person of worth, at least on an equal basis with others	1	2	3	4
I wish I could have more respect for myself	1	2	3	4
All in all, I am inclined to feel that I am a failure	1	2	3	4
I take a positive attitude toward myself	1	2	3	4

Appendix 13 IPIP Neuroticism Scale

INSTRUCTIONS

Please read the following statements carefully and indicate the extent to which you agree each statement describes **you** . Please answer as honestly as you can. Your responses will be treated as confidential. Circle only one answer for each question.

	Very Accurate	Moderately Accurate	Neither Accurate nor Inaccurate	Moderately Inaccurate	Very Inaccurate
I Seldom feel blue.	5	4	3	2	1
I am often down in the dumps.	5	4	3	2	1
I rarely get irritated.	5	4	3	2	1
I am not easily bothered by things.	5	4	3	2	1
I have frequent mood swings.	5	4	3	2	1
I feel comfortable with myself.	5	4	3	2	1
I am filled with doubts about things.	5	4	3	2	1
I am not easily frustrated.	5	4	3	2	1
I feel threatened easily.	5	4	3	2	1
I fear for the worst.	5	4	3	2	1
I seldom get mad.	5	4	3	2	1
I often feel blue.	5	4	3	2	1
I worry about things.	5	4	3	2	1
I dislike myself.	5	4	3	2	1
I am relaxed most of the time.	5	4	3	2	1
I remain calm under pressure.	5	4	3	2	1
I panic easily.	5	4	3	2	1
I am very pleased with myself.	5	4	3	2	1
I get stressed out easily.	5	4	3	2	1
I rarely lose my composure.	5	4	3	2	1

Appendix 14 Toronto Alexithymia Scale

Date …… **Participant ID ……....**

Below are a number statements that people may make about themselves. Please read each statement and then draw a vertical line through the response below it that most closely describes how much you agree with the statement.

- **I am often confused about what emotion I am feeling.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **It is difficult for me to find the right words for my feelings.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I have physical sensations that even doctors do not understand.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I am able to describe my feelings easily.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I prefer to analyse problems rather than just describe them.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **When I am upset, I do not know if I am sad, frightened or angry.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I am often puzzled by sensations in my body.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I prefer to just let things happen rather than to understand why they turned out that way.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I have feelings that I cannot quite identify.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **Being in touch with emotions is essential.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I find it hard to describe how I feel about people.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **People tell me to describe my feelings more.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I do not know what is going on inside me.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I often do not know why I am angry.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I prefer talking to people about their daily activities rather than their feelings.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I prefer to watch "light" entertainment shows rather than psychological dramas.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **It is difficult for me to reveal my innermost feelings, even to close friends.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I can feel close to someone, even in moments of silence.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

- **I find examination of my feelings useful in solving personal problems.**

[strongly disagree] [disagree] [neither agree nor disagree] [agree] [strongly agree]

Appendix 15 Spielberger Trait Anxiety Scale

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

		ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
1.	I feel pleasant	1	2	3	4
2.	I feel nervous and restless	1	2	3	4
3.	I feel satisfied with myself	1	2	3	4
4.	I wish I could be as happy as others seem to be	1	2	3	4
5.	I feel like a failure	1	2	3	4
6.	I feel rested	1	2	3	4
7.	I am "calm, cool, and collected"	1	2	3	4
8.	I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
9.	I worry too much over something that really doesn't matter	1	2	3	4
10.	I am happy	1	2	3	4
11.	I have distrubing thoughts	1	2	3	4
12.	I lack self-confidence	1	2	3	4
13.	I feel secure	1	2	3	4
14.	I make decisions easily	1	2	3	4
15.	I feel inadequate	1	2	3	4
16.	I am content	1	2	3	4
17.	Some unimportant thought runs through my mind and bothers me	1	2	3	4
18.	I take disappointments so keenly that I can't put them out of my head	1	2	3	4
19.	I am a steady person	1	2	3	4
20.	I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

Appendix 16 Cook-Medley Hostility Scale

Please circle the response option that best represents your thoughts.
Please be as HONEST and FRANK as possible.

		TRUE	FALSE
1	When I take a new job, I like to be tipped off on who should be gotten next to.	T	F
2	When someone does me a wrong I feel I should pay him back if I can, just for the principle of the thing.	T	F
3	I prefer to pass by school friends, or people I know but have not seen for a long time, unless they talk to me first.	T	F
4	I have often had to take orders from someone who did not know as much as I did.	T	F
5	I think a great many people exaggerate their misfortune in order to gain the sympathy and help of others.	T	F
6	I think most people would lie to get ahead.	T	F
7	Someone has it in for me.	T	F
8	Most people are honest chiefly through fear of being caught.	T	F
9	Most people will use somewhat unfair means to gain profit or an advantage rather than lose it.	T	F
10	I commonly wonder what hidden reason another person may have for doing something nice for me.	T	F
11	It makes me impatient to have people ask my advice or otherwise interrupt me when I am working on something important.	T	F
12	I feel that I have often been punished without cause.	T	F
13	I am against giving money to beggars.	T	F
14	Some of my family have habits that bother and annoy me very much.	T	F
15	My relatives are nearly all in sympathy with me.	T	F
16	My way of doing things is apt to be misunderstood by others.	T	F
17	I don't blame anyone for trying to grab everything he can in this world.	T	F
18	No one cares much what happens to you.	T	F
19	I can be friendly with people who do things which I consider wrong.	T	F
20	It is safer to trust nobody.	T	F
21	I do not blame a person for taking advantage of someone who lays himself open to it.	T	F
22	I have often felt that strangers were looking at me critically.	T	F
23	Most people make friends because friends are likely to be useful to them.	T	F
24	I am sure I am being talked about.	T	F
25	I am likely not to speak to people until they speak to me.	T	F

26	Most people inwardly dislike putting themselves out to help other people.	T	F
27	I tend to be on my guard with people who are somewhat more friendly than I had expected.	T	F
28	I have sometimes stayed away from another person because I feared doing or saying something that I might regret afterwards.	T	F
29	People often dissappoint me.	T	F
30	I like to keep people guessing what I'm going to do next.	T	F
31	I frequently ask people for advice.	T	F
32	I am not easily angered.	T	F
33	I have often met people who were supposed to be experts who were no better than I.	T	F
34	I would certainly enjoy beating a crook at his own game.	T	F
35	It makes me feel like a failure when I hear of the success of someone I know well.	T	F
36	I have at times had to be rough with people who were rude or annoying.	T	F
37	People generally demand more respect for their own rights than they are willing to allow for others.	T	F
38	There are certain people whom I dislike so much that I am inwardly pleased when they are catching it for something they have done.	T	F
39	I am often inclined to go out of my way to win a certain point with someone who has opposed me.	T	F
40	I am quite often not in on the gossip and talk of the group I belong to.	T	F
41	The man who had most to do with me when I was a child (such as my father, step-father, etc.), was very strict with me.	T	F
42	I have often found people jealous of my good ideas, just because they had not thought of them first.	T	F
43	When a man is with a woman he is usually thinking about things related to her sex.	T	F
44	I do not try to cover up my poor opinion or pity of a person so that he won't know how I feel.	T	F
45	I have frequently worked under people who seem to have things arranged so that they get credit for good work but are able to pass off mistakes onto those under them.	T	F
46	I strongly defend my own opinions as a rule.	T	F
47	People can pretty easily change me even though I thought that my mind was already made up on a subject.	T	F
48	Sometimes I am sure that other people can tell me what I'm thinking.	T	F
49	A large number of people are guilty of bad sexual conduct.	T	F
50	It takes a lot of argument to convince most people of the truth.	T	F

Appendix 17 Social-evaluative Threat VAS

Date: ..... Participant ID: _ _ _ _ _ _ _ _

INSTRUCTIONS

Please read the statements below and indicate how much the statements reflect how you feel about your upcoming performance by making a mark on each line.

It is very important to me that I give a good performance

Totally disagree _____ Totally agree

The outcome of the performance is not crucial to my self esteem

Totally disagree _____ Totally agree

I am confident I will give a good performance

Totally disagree _____ Totally agree

I worry that the judges and other performers will judge me negatively if I give a poor performance

Totally disagree _____ Totally agree

I worry about things outside of my control affecting my performance

Totally disagree _____ Totally agree

How good my performance is will not affect what other people think of me

Totally disagree _____ Totally agree

I am in control of my performance and I have the talent to perform well

Totally disagree _____ Totally agree

Appendix 18 Social-evaluative Threat and General Stress VAS

INSTRUCTIONS

Please read the statements below and indicate how much the statements reflect how you feel about your upcoming performance and general feelings by making a mark on each line.

I worry about my general health

Totally disagree _____ Totally agree

It is very important to me that I give a good performance

Totally disagree _____ Totally agree

I worry about things outside of the audition

Totally disagree _____ Totally agree

I worry about getting to the venue on time

Totally disagree _____ Totally agree

I worry about things outside of my control affecting my performance

Totally disagree _____ Totally agree

The outcome of the performance is not crucial to my self esteem

Totally disagree _____ Totally agree

I am confident I will give a good performance

Totally disagree _____ Totally agree

I worry that the judges and other performers will judge me negatively if I give a poor performance

Totally disagree _____ Totally agree

I worry about the venue

Totally disagree _____ Totally agree

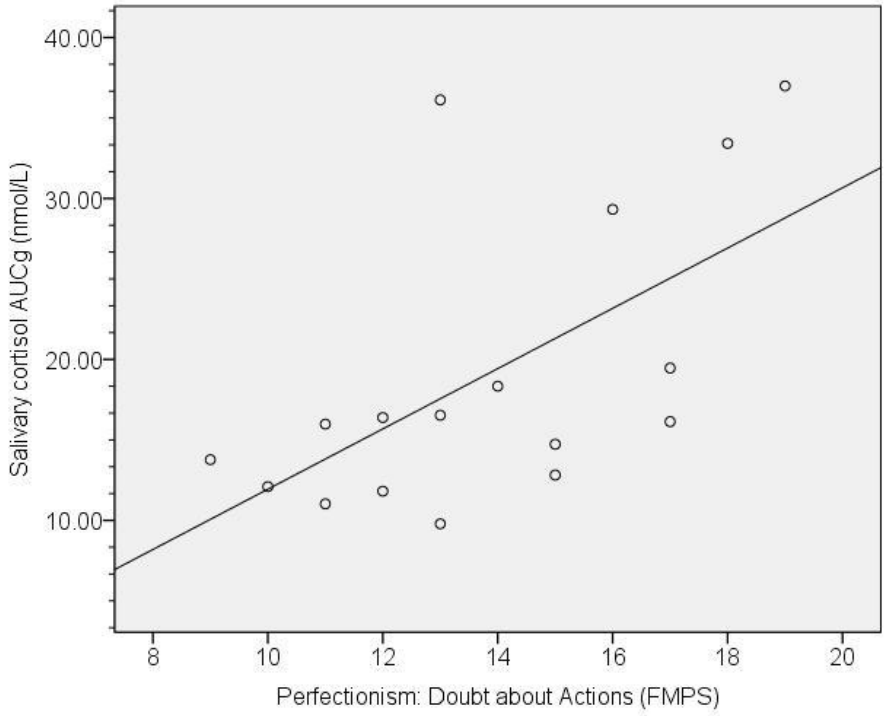
How good my performance is will not affect what other people think of me

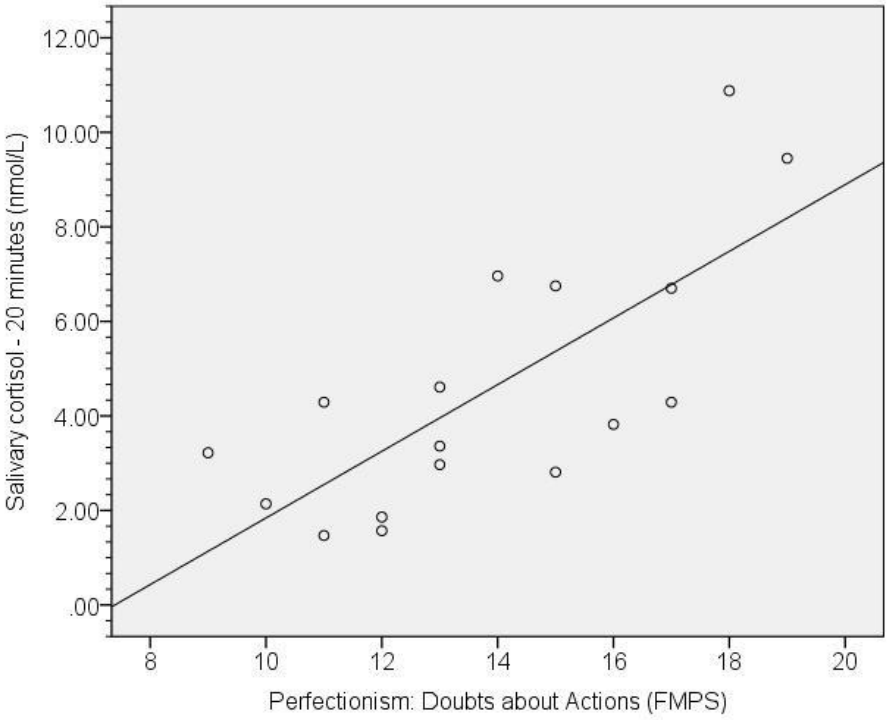
Totally disagree _____ Totally agree

I am in control of my performance and I have the talent to perform well

Totally disagree _____ Totally agree

Appendix 19 Scatterplots of Perfectionism: Doubts about Actions and salivary cortisol AUCg and measurement at - 20 minutes relative to audition onset (nmol/L)





Appendix 20 Cognitive Distraction/Interference VAS – Control and Stress Versions

Control visit

Participant .....

How distracted by your thoughts did you feel while you performed the previous memory tasks?

Not at all _____ A lot

How much did you feel yourself intentionally suppressing off-task thoughts while you performed the previous memory tasks?

Not at all _____ A lot

How distracted did you feel by thoughts about the test scenario while performing the memory tasks?

Not at all _____ A lot

How much did you feel yourself intentionally suppressing off task thoughts about the test scenario while you were performing the memory tasks?

Not at all _____ A lot

Stressvisit

Participant .....

How distracted by your thoughts did you feel whilst you performed the previous memory tasks?

Not at all _____ A lot

How much did you feel yourself intentionally suppressing off-task thoughts whilst you performed the previous memory tasks?

Not at all _____ A lot

How distracted did you feel by thoughts about the challenging tasks whilst performing the memory tests?

Not at all _____ A lot

How much did you feel yourself intentionally suppressing off-task thoughts about the challenging tasks whilst you were performing the memory tests?

Not at all _____ A lot

Appendix 21 Stress protocol – Combined psychosocial and physical stressor

General protocol

Researchers

Researcher 1: Responsible for the general running of the stress protocol. Main responsibilities are showing participants in and out of the stress induction room and reading the instructions before the speech and cold pressor tasks.

Researcher 2: Panel member. Reads out standardised instructions to the participants if they falter during the speech task and controls timing.

Researcher 3: Panel member.

Procedure

Participant is shown into the stress induction room by researcher 1. The stress induction room is laid out in accordance to Figure 1.

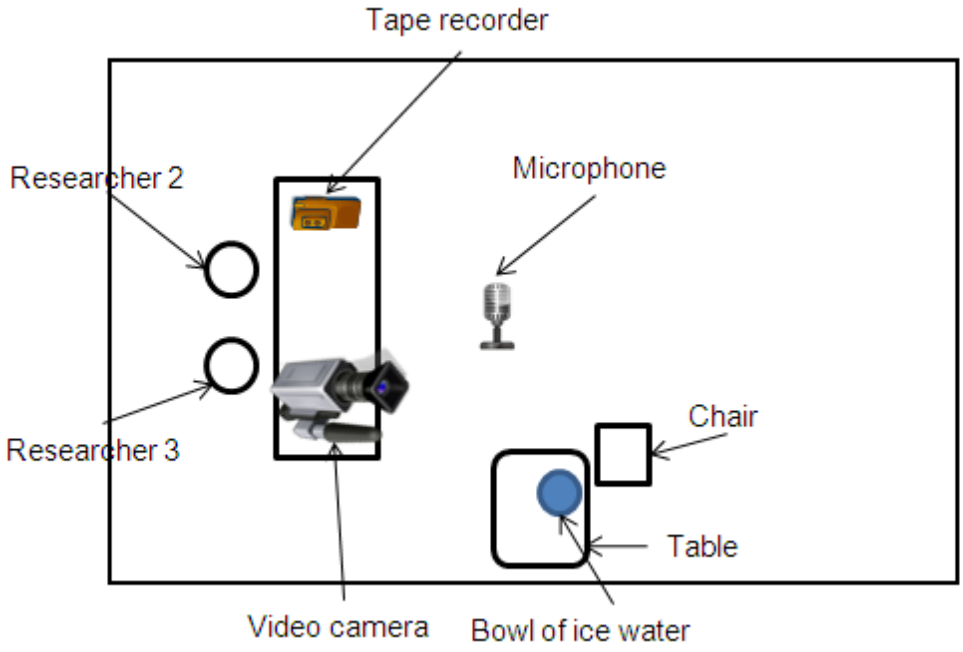


Figure 1. Stress induction room layout.

Researcher 1 directs the participant to stand in front of the microphone in the centre of the room and reads Instruction Sheet 1:

"Good afternoon. I will now explain today's task to you. We would like you to assume the role of a job applicant who has been invited to a personal interview with the committee for a position as a management trainee within the department. You will be given 5 minutes to prepare a speech of five minutes duration to present to the committee members explaining why you feel you are a good applicant for the job. Your speech will be filmed and video recorded for voice frequency analysis and assessment of non-verbal behaviour. After the speech you will be asked to complete a secondary task which will be explained after you have delivered your speech.

Are you happy to continue with the procedure? Do you have any questions"?

Researcher 1 directs the participant to a second room (testing cubicle) and provides a pen and paper for preparatory notes. Just before returning to the stress induction room salivary cortisol, blood pressure/heart rate and subjective measures are collected by researcher 1.

Researcher 1 takes the participant back into the stress induction room and directs them to stand in front of the microphone. Researcher 1 leaves the room. Researcher 2 assumes responsibility for the stress induction and is responsible for keeping to the study time schedule. Researcher 2 switches on the audio and video recording equipment and reads Instruction Sheet 2:

"Good afternoon. You have five minutes to give your presentation starting from now. Please begin."

The panel are required to observe the participant throughout their speech (taking occasional notes). Panel members must remain non-communicative and provide no verbal or non-verbal prompts or reassurance. If the participant stops talking the panel must wait in silence observing the participant for 20 seconds. If the participant remains silent Researcher 2 waits 20 seconds again and can give further standardised prompts (shown on Information Sheet 2:

"You still have time, please continue"

If needed further prompts can be given until the 5 minutes has elapsed:

"Why do you think you are better than other applicants for this position?"

"What would you do if your application here was unsuccessful?"

"Tell us about a difficult obstacle you have faced and overcome in a previous employment role"

Once 5 minutes has passed Researcher 2 must interrupt the participant and tell them 5 minutes is up. Researcher 2 turns of the video and audio equipment and motions to Researcher 1 that it is time to re enter the stress induction room.

Researcher 1 sits the participant down at the table and takes blood pressure and heart rate measures.

Researcher 1 reads Instruction Sheet 3 to the participant:

"For the physically challenging task we will ask you to submerge you hand up to your wrist in this bowl of ice water. Whilst you have your hand in the water the panel will observe your behaviour and we will video record you for facial expression analysis. Please look into the camera whilst your hand is in the water and keep your hand submerged for as long as you can".

"Are you happy to continue with the task? Do you have any questions?"

Researcher 2 switches on the video and audio recorders. Researcher 1 direct the participant to submerge their hand into the water:

"When you are ready please place your hand into the bowl water until the water is above your wrist. Please keep your hand in the water as long as you can whilst looking into the camera"

Researcher 2 is responsible for timing and recording the length of time the participant has their hand submerged. After 3 minutes all participants are asked to remove their hand from the water.

The stress induction procedure ends. Researcher 1 takes the participant back to the testing cubicle to commence cognitive testing.

Table 1. Stress induction procedure.

Time (minutes)	Event
0	Participant shown into the stress induction room. Instruction Sheet 1 is read to the participant by researcher 1
2	Participant is taken to testing cubicle and given 5 minutes to prepare for public speaking task (BP/HR)
7	Participant returns to stress induction room. Researcher 2 reads Instruction Sheet 2 and participant presents 5 minute speech to panel. Researcher 2 follows directives on Instruction Sheet 2 if participant pauses or stops presenting
12	One minute recovery & BP/HR, cortisol measure
15	Instruction Sheet 3 read by researcher 1. Participant places hand in cold water bowl (BP/HR at end of task)
18	End of stress induction

Considerations for second stress exposure

The panel and Researcher 1 should changed across stress induction visits.

The subject of the public speaking task should also be changed across the visits. Instruction Sheet 1b should be read to participants on the second visit:

"Good afternoon. I will now explain today's task to you. We would like you to assume the role of a job applicant who has been invited to a personal interview with the panel to apply for a job as a research assistant on a project studying attitudes to gambling. You will be given 5 minutes to prepare a speech of five minutes duration to present to the panel explaining why you feel you would be a good applicant for the position. Your speech will be filmed and video

recorded for voice frequency analysis and assessment of non-verbal behaviour. After the speech you will be asked to complete a secondary task which will be explained after you have delivered your speech.

Are you happy to continue with the procedure? Do you have any questions"?

Appendix 22 Intervention drink intake dairy (Study 4)

Drink information

- Consume one carton in the morning every day
- Drinks must be kept in the fridge
- Complete your drink diary every day ensuring you give any detail of missed drinks (including explanation) and medication taken etc...
- You must consume your drink in the morning but if you forget you may take it later. It is important that this does not happen often and you make a record of when it does
- Please do not heat the drinks or add them to hot drinks.
- Ensure you arrange a restock visit to pick up more drinks before you run out by emailing or phoning the contact details provided.

Remember to bring your unused drinks back in the cool bag with you when restocking

TEST DIARY1

UNIVERSITY OF LEEDS

If found, please return to:

Neil Boyle
Institute of Psychological Sciences
University of Leeds
Leeds
LS2 9JT

Phone: 0113 343 5753
Email: pscchals@leeds.ac.uk

Participant number _____

Drink code _____

Day 1

Date: _____/_____/ _____

Remember to drink:

One 250 ml carton of milk every MORNING

Drink taken: Yes ☐ No ☐

Time of consumption: _____am/pm

If you did not take the drink today please say why below. If you feel unwell, or take any medication (including over-the-counter medication such as aspirin) please record in the boxes below. Otherwise please leave blank.

Adverse Events

Medication taken:

N.B. The date on the drinks is the manufacture date, not use by date.

Day

Date: _____/_____/ _____

Remember to drink:

One 250 ml carton of milk every MORNING

Drink taken: Yes ☐ No ☐

Time of consumption: _____am/pm

If you did not take the drink today please say why below. If you feel unwell, or take any medication (including over-the-counter medication such as aspirin) please record in the boxes below. Otherwise please leave blank.

Adverse Events

Medication taken:

N.B. The date on the drinks is the manufacture date, not use by date.

Below are some statements about feelings and thoughts.
Please circle the box that best describes your experience of each over the last week

	None of the time	Rarely	Some of the time	Often	All of the time
1. I've been feeling optimistic about the future	1	2	3	4	5
2. I've been feeling useful	1	2	3	4	5
3. I've been feeling relaxed	1	2	3	4	5
4. I've been feeling interested in other people	1	2	3	4	5
5. I've had energy to spare	1	2	3	4	5
6. I've been dealing with problems well	1	2	3	4	5
7. I've been thinking clearly	1	2	3	4	5
8. I've been feeling good about myself	1	2	3	4	5
9. I've been feeling close to other people	1	2	3	4	5
10. I've been feeling confident	1	2	3	4	5
11. I've been able to make up my own mind about things	1	2	3	4	5
12. I've been feeling loved	1	2	3	4	5
13. I've been interested in new things	1	2	3	4	5
14. I've been feeling cheerful	1	2	3	4	5

Day 4

Date: _____/_____/ _____

Remember to drink:
One 250 ml carton of milk every MORNING

Drink taken: Yes ☐ No ☐

Time of consumption: _____am/pm

If you did not take the drink today please say why below. If you feel unwell, or take any medication (including over-the-counter medication such as aspirin) please record in the boxes below. Otherwise please leave blank.

Adverse Events

Medication taken:

N.B. The date on the drinks is the manufacture date, not use by date.

Appendix 23 Mean subjective mood ratings (POMS) across stress visit and condition in dimensions without significant differences across or between the response profiles (study4)

	Condition	Stress visit	Measurement time points (minutes)							
			0		+ 10		+ 35		+ 45	
			Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
POMS										
Depression-dejection (0 - 60)	182	1	**3.62**	1.07	**2.92**	1.07	**4.24**	1.37	**4.52**	1.41
		2	**3.62**	1.18	**4.31**	1.43	**3.54**	1.21	**3.38**	1.25
	375	1	**1.68**	0.69	**1.28**	0.60	**2.56**	0.70	**2.00**	0.64
		2	**2.20**	1.12	**1.88**	0.73	**2.58**	1.33	**1.27**	0.48
Anger-hostility (0 - 48)	182	1	**2.46**	0.72	**2.85**	1.07	**3.84**	1.10	**3.48**	1.11
		2	**1.50**	0.47	**2.08**	0.71	**1.62**	0.53	**1.81**	0.58
	375	1	**2.44**	0.70	**1.68**	0.61	**4.04**	1.16	**3.96**	1.47
		2	**2.24**	0.84	**1.92**	0.76	**2.92**	1.23	**1.77**	0.40
Fatigue-inertia (0 - 28)	182	1	**2.96**	0.64	**2.96**	0.74	**2.80**	0.68	**3.72**	0.82
		2	**3.58**	0.79	**3.73**	0.91	**2.50**	0.60	**3.19**	0.67
	375	1	**3.08**	0.51	**2.48**	0.45	**2.15**	0.37	**2.96**	0.63
		2	**4.16**	0.85	**3.33**	0.62	**2.88**	0.62	**2.92**	0.63
Confusion-bewilderment (0 - 28)	182	1	**4.54**	0.39	**4.46**	0.43	**6.40**	0.79	**6.24**	0.82
		2	**5.81**	0.73	**6.34**	0.85	**4.73**	0.61	**5.08**	0.84
	375	1	**4.24**	0.33	**3.64**	0.34	**5.56**	0.47	**4.81**	0.63
		2	**4.68**	0.42	**4.54**	0.45	**4.15**	0.53	**4.04**	0.44

POMS - Profile of Mood States