# CORRELATIONS BETWEEN ANXIETY, CHILDHOOD TRAUMA, RESILIENCE, AND PHYSIOLOGICAL DETERMINENTS OF HEALTH, BOTH CENTRALLY AND PERIPHERALLY, IN ADOLESCENTS

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**Dissertation presented for the Degree of Doctor of Physiological**

**Sciences at the Stellenbosch University**

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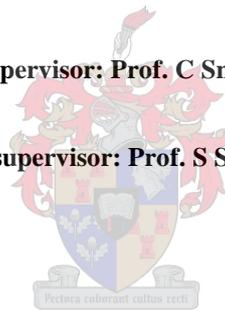
**March 2016**

**"If someone comes along and shoots an arrow into your heart, it's fruitless to stand there and yell at the person. It would be much better to turn your attention to the fact that there's an arrow in your heart..."**

— Pema Chödrön, Start Where You Are: A Guide to Compassionate Living

**Declaration**

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**ABSTRACT**

Anxiety disorders are among the most prevalent of psychiatric disorders across age groups, with onset typically in childhood or early adolescence, and risk for developing an anxiety disorder increasing with trauma/childhood maltreatment. Little is known about biomarkers of resilience/vulnerability in relation to subclinical anxiety, especially when trauma-exposed adolescents are implicated. Therefore, better elucidation of the neuro-endocrine and -immunological underpinnings relative to anxiety and trauma, may highlight specific avenues to target with more effective diagnosis, monitoring and/or treatment strategies in the context of youth at risk for later development of anxiety disorders. Thus, our aims were to elucidate the central and peripheral neuroendocrine and immunological profiles in association with anxiety proneness, in comparison to childhood trauma, in older adolescents, and to assess potential outcome modulators.

A total of 43 participants, aged 15-18, were selected from an initial cohort of 1149 adolescents. Participants were delineated into four groups based on levels of anxiety proneness and trauma exposure, using questionnaires and a structured diagnostic interview. Blood obtained from each participant was analysed for an HPA-axis hormone profile (cortisol, prolactin, testosterone and dehydroepiandrosterone-sulphate (DHEAs) and immune status (total white blood cell count, leukocyte glucocorticoid receptor (GR) expression and serum cytokine and myeloperoxidase (MPO) levels). Resilience (coping capacity), self-esteem and handedness were assessed *via* questionnaires. Verbal- and visuospatial working memory, as well as executive neurocognitive function, were assessed by means of the administration of neurocognitive tests. A structural Magnetic Resonance Imaging (MRI) was performed to determine left *versus* right grey matter volumes of the thalamus, amygdala, hippocampus, and Prefrontal cortex (PFC). Finally, HPA-axis responsivity and concurrent state anxiety to an *in vivo* Bexamethasone suppression test, in conjunction with as a psychosocial stress test (TSST), were assessed.

In terms of neurophysiological maladaptations, main findings included a relatively larger association with anxiety proneness, compared to childhood maltreatment. Specifically, anxiety proneness was associated with poorer neurocognitive function, increased right amygdala volume, lower serum DHEAs levels, lower peripheral leukocyte counts, and increased GR expression. In terms of potential outcome modifying factors (OMFs), resilience and self-esteem were affected by trauma, but not anxiety proneness, while a higher degree of right handedness was associated with poorer neurophysiological outcomes. Furthermore, increased serum IL-12p70 and MPO (suggesting relatively more pro-inflammatory state) were associated with anxiety scales and

emotional/physical abuse. Also, better PFC neurocognitive function and larger left PFC volumes were associated with better physiological outcome as indicated by levels of GR expression and DHEAs.

In conclusion, this is the first study to have investigated neurophysiological adaptations, as well as psycho-physiological responses to HPA-axis suppression and a psychosocial stress test, in association with anxiety proneness and trauma exposure, in adolescents of low socio-demographic background. Results suggest for the study population, a) chronic hypo-activity and acute hypo-reactivity of the lower HPA-axis, b) neurophysiological perturbations associated relatively closely with anxiety proneness, when compared to trauma exposure, c) central correlates associated with physiological outcome, and d) a higher degree of consistent right handedness to be a potential marker of vulnerability in terms of neurophysiology and anxiety.

**OPSOMMING**

Angsversteurings is van die mees algemeenste van al die psigiatrise afwykings oor al die ouderdomsgroepe heen, met die aanvang gewoonlik tydens kindertyd of vroë adolessensie, en met die risiko om n angsversteuring te ontwikkel toenemend met kinder trauma/mishandeling. Min is tans bekend aangaande biomerkers van weerstandigheid/kwesbaarheid in verhouding tot sub-kliniese angs, veral wanneer trauma-blootgestelde adolessente ge-impliseer is. Daarom sal beter toeligting van die neuro-endokriene en –immuun merkers, relatief tot angs en trauma, mag bydra tot die ontwikkeling van meer effektiewe voorkoming, monitering en/of behandeling strategieë in die konteks van jeug wat die risiko loop vir latere ontwikkeling van angsversteurings. Ons doelstelling was om die sentrale en perifere neuro-endokriene en immunologiese profiele wat ge-assosieer is met angsvatbaarheid , in teenstelling met kinder trauma, in ouer adolessente te bepaal, en om ook potensiële gevolge modulerende (OMFs) te bepaal.

'n Totaal van 43 deelnemers, ouderdom tussen 15 en 18 jaar, was geselekteer uit n aanvanklike kohort van 1149 adolessente. Deelnemers is ingedeel in vier groepe gebasseer op vlakke van angsvatbaarheid en kinder trauma, deur gebruik te maak van vraelyste en 'n gestruktureerde diagnostisie onderhoud. Bloed monsters van elke deelmener was ge-analiseer vir 'n HPA-axis hormoon profiel (kortisol, prolaktien, testosteroon en dehidroepiandrosteroon-sulfaat (DHEAs) en immuun status (totale witbloedsel telling, leukosiet glucocorticoid reseptor (GR) uitdrukking en serum sitokien en myeloperoxidase (MPO) vlakke). Weerstandigheids (uithouvermoë), selfwaarde/selfvertroue, en handedness was bepaal via vraelyste. Verbale- en visieruimtelike werkende geheue, asook uitvoerbare neurokognitiewe funksie was bepaal deur middel van neurokognitiewe toetse. 'n Strukturele Magnetiese Resonans Skandering (MRS) was verrig om links versus regs grysstof volumes van die thalamus, amygdala, hippocampus en prefrontale korteks (PFK) te bepaal. Laastens, HPA-axis reaksie kapasiteit en gesamentlike toestand angs as n gevolg van 'n Bexamethasone onderdrukking toets, in kombinasie met n psigososiale stres toets (TSST), is vasgestel.

In terme van neurofisiologiese wanaanpassings, sluit primêre bevindinge in 'n relatiewe groter assosiasie met angsvatbaarheid, in vergelyking met kinder trauma. Meer spesifiek, angsvatbaarheid was ge-assosieer met swakker neurokognitiewe funksies, vergrote regter amygdala volume, laer serum DHEAs vlakke, laer periferale leukosiete tellings en verhoogde GR uitdrukking. In terme van potensiele OMFs, was weerstandigheid en selfwaarde/selfvertroue ge-affekteer deur trauma en nie angsvatbaarheid nie, terwyl n hoër graad van regshandigheid ge-assosieer was met swakker neurofisiologiese resultate. Vervolgens was verhoogde serum IL-

12p70 en MPO (wat n relatiewe meer pro-inflammatoriese toestand suggereer), ge-assosieer met hoër angs eindtellings en emosionele/fisiese mishandeling. Verder was beter PFK neurokognitiewe funksie en groter linker PFK volumes ge-assosieer met beter fisiologiese gevolge, aangedui deur vlakke van GR uitdrukking en DHEAs.

Ter afsluiting, die huidige studie is die eerste om neurofisiolgiese aanpassings te bestudeer, asook psigofisiologiese reaksies tot HPA-axis onderdrukking en 'n psigososiale stres toets, in verband met angsvatbaarheid en kinder trauma, in adolessente met lae sosio-demografiese agtergronde. Die resultate vir die huidige studie populasie suggereer, a) kroniese hipo-aktiwiteit en kortstondige hipo-reaktiwiteit van die laer HPA-axis, b) neurofisiologiese verstorings relatief hoog ge-assosieer met angs vatbaarheid, in vergelyking met kinder trauma, c) sentrale merkers ge-assosieer met fisiolgiese gevolge, en d) a hoër graad van konstante regs handigheid wat n potensiële merker van vatbaarheid kan wees, in terme van neurofisiologie en angs.

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## ACKNOWLEDGEMENTS

I would like to thank the following people for their contributions to this thesis:

- First of all, I would like to acknowledge my primary supervisor, Prof C Smith, for all her guidance, support, and involvement on so many levels. It has been an honour to be her student as she has always allowed for freedom to explore novel avenues, while providing ample assistance.

- My appreciation also goes to my co-supervisor, Prof S Seedat, for providing opportunities that made the current thesis possible, specifically those enabling research on a multi-disciplinary level.

- I would like to thank Ms L Martin for participating in this joint undertaking with me, particularly for assisting with the recruitment, screening, conducting of scans, and neurocognitive assessments.

- I offer my obeisance to the Higher Power through which the execution of this degree has provided immense growth, satisfaction and meaning.

- Last but not least, I would like to express gratitude to my beloved and my family, for all the support, perspective and encouragement.

I would also like to thank the following people for technical assistance:

- Letticia Busiswa, for assisting in communicating with schools and participants, for helping with fetching participants form their homes, and for performing the blood draws.

- Rozanne Adams, for flow cytometry procedures.

- Melanie Bishop and Dr Nortje for assisting with the Trier Social Stress Test.

- Prof M Kidd for assisting with the statistical analysis.

- Luan Africa, for isolating neutrophils and peripheral blood mononuclear cells for future analysis.

- Fatima, for analysing the structural MRI data.

- Ahswin Isaacs, for full blood counts.

- Pathcare laboratories, for analysis of blood samples for serum cortisol, testosterone, DHEAs, prolactin and ACTH.

**LIST OF RELEVANT PUBLICATIONS AND CONGRESS CONTRIBUTIONS**

**Peer-Reviewed Paper**

Martin L, Viljoen, M, Kidd M, Seedat, S. Are childhood trauma exposures predictive of anxiety sensitivity in school attending youth? Journal of Affective Disorders 168 (2014) 5–12

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**LIST OF ABBREVIATIONS**

ACTH            adrenocorticotrophic hormone

A-COPE          Adolescent Coping Orientation for Problem Experiences

ANOVA           analysis of variance

AS              anxiety sensitivity

AUDIT           Alcohol Use Disorders Identification Test

BAS             behavioural activation/approach system

BIS             Behavioural Inhibition System

CASI            Child Anxiety Sensitivity Index

CES-DC          Center for Epidemiological Studies Depression Scale for children

CD              cluster of differentiation

CD-RISC         Connor-Davidson Resilience Scale

CD45RO          marker for helper T memory cells

CH              consistent/strong-handers

CHPC            centre for high performance computing

CNS             central nervous system

CRH             corticotrophin releasing hormone

CSF             cerebrospinal fluid

CTQ             The Childhood Trauma Questionnaire

CUBIC           Cape Universities Brain Imaging Centre

Dex             Dexamethasone

| | |
|---|---|
| DHEAs | dehydroxyepiandrosterone-sulphate |
| DLPFC | dorsolateral prefrontal cortex |
| DSM | Diagnostic and Statistical Manual of Mental Disorders |
| DST | dexamethasone suppression test |
| DTI | Diffusion Tensor Imaging |
| DUDIT | The Drug Use Disorders Identification Test |
| EEG | electroencephalogram |
| EDTA | ethylenediaminetetraacetic acid |
| EHI | Edinburgh Handedness Inventory |
| ELISA | enzyme-linked immunosorbent assay |
| ELS | early life stress |
| FITC | fluoresceinisothiocyanate |
| FFFS | fight–flight–freeze system |
| GABA | gamma-aminobutyric acid |
| GC | glucocorticoid |
| GR | glucocorticoid receptor |
| HPA-axis | hypothalamo-pituitary-adrenal axis |
| HPG-axis | hypothalamic-pituitary-gonadal axis |
| IAPS | International Affective Processing Scale task |
| IFϒ | interferon γ |
| IL | Interleukin |

| | |
|---|---|
| ICD | International Classification of Diseases |
| ICH | inconsistent/mixed-handers |
| Ig | Immunoglobulin |
| LC/NE | Locus Ceruleus Norepinephrine |
| LPS | lipopolysaccharide |
| LSD | Fisher least significant difference |
| LTP | long term potentiation |
| MINI-KID | Neuropsychiatric Interview-Kid for children and adolescents |
| MHC | major histocompatibility complex |
| MoAb | monoclonal antibodies |
| MR | mineralocorticoid receptor |
| MRI | Magnetic Resonance Imaging |
| NIMH | National Institute of Mental Health |
| NFκβ | nuclear factor-kappa beta |
| NK | natural killer lymphocyte |
| OMF | outcome modulating factors |
| PE | phycoerythrin |
| PBMC | peripheral blood mononuclear cell |
| PTSD | Post Traumatic Stress Disorder |
| PFC | Prefrontal cortex |
| PK | psychokinesis |

| | |
|---|---|
| PVN | paraventricular nucleus |
| RDoC | Research Domain Criteria |
| R-SES | Rosenberg Self-esteem Scale |
| RST | Reinforcement Sensitivity Theory |
| SAM | sympatho-adrenal medullary |
| SANC | South African Nursing Council |
| SD | standard deviation |
| SEM | standard error of the mean |
| SSAIS | Senior South African Individual Scale (revised) |
| SST | serum separation tubes |
| STAI | State-Trait Anxiety Inventory |
| TCR | T cell receptor |
| Tc | T cytotoxic cell |
| Th | T helper cell |
| TOL | Tower of London |
| TSST | Trier Social Stress Test |
| VAS | Visual Analogue Scale |
| VLPFC | ventrolateral prefrontal cortex |
| WM | working memory |
| WMS-IR | Wechsler memory scale (Revised) Immediate Recall |
| WBC | white blood cell |

# CHAPTER 1

# INTRODUCTION

A growing body of evidence is pointing towards the idea that both cause and cure of psychopathology, along with accompanying physiological perturbations, reside within the mind. The term psychokinesis (PK) has been ascribed to the phenomenon whereby one's state of consciousness can modify physical properties of matter; specifically one's physiology. Given the possible implications of PK in biological healing, it is not surprising that this phenomenon has captured the attention of many great scholars, spanning the disciplines of psychology, particle physics, neurophysiology, cell biology and others.

Simply attempting to define metaphysical notions such as "the mind" and "consciousness" in itself proves to be a problematic endeavour, given the different reference frameworks underlying the different fields of studies. Nevertheless, regardless of whether the mind is seated within the brain or whether the mind is the brain, one's reality – based on beliefs about the self as well as beliefs about the self in relation to the external world – seems to be pivotal in determining overall health (Helm, et al., 2000; Matthews, et al., 1998). From a neurophysiological point of view, stimuli are processed within the contexts of these unconscious neural pathways and certain immune and endocrine parameters in circulation appear to be at the interface between the conditioned perceptions of stimuli and their physiological sequelae, in effect providing a connection between mind and body.

Interestingly, within the contexts of childhood maltreatment and anxiety proneness, some individuals escape the development of psychopathology in the face of extreme adverse conditions. We postulated that this phenomenon demonstrates individual differences in the processing of stressful stimuli, accompanied by differences in adaptation of psychoneuroendocrine systems to these stressors. Given the interconnected nature of the neuroendocrine and immune systems, this may also impact on immune competence – this field however has not received much attention by researchers to date. We therefore postulated that pathological stress stimuli processing, crystallised in neural pathways over the course of childhood and adolescence, encompasses three levels: a) the environment, b) personality dimensions, and c) the interaction of (a) and (b) which produces neurophysiological maladaptations. Effective intervention strategies would therefore either address all three levels, or at least intercept at the level identified as the largest contributor to pathology. However, before such attempts at fine-tuning intervention can be made, key variables representative of the three levels outlined above need to be identified.

Thus, in order to devise more effective intervention strategies - specifically in the context of anxiety featuring in the South African adolescent population, in a low socio-economic setup - identification of the relative contributions of a) childhood maltreatment (the environmental context) *versus* b) anxiety proneness (a key personality dimension), as well as the identification of  c) neurophysiological markers of maladaptation (as a consequence of interaction of a) and b)), warrants investigation.  Once a distinction between childhood trauma and anxiety proneness has been made, in terms of relative contributors to psycho-neurophysiological aberrations, and the latter profiles been delineated, this knowledge can be applied in the identification, monitoring and/or managing of at risk populations over time, by means of conventional or alternative medicine – this may take the form of conventional pharmacological or natural medicine supplementation, psychological intervention and/or practices following Eastern traditions, such as mindfulness-based training, depending on the most relevant therapeutic target in a given situation/individual.

Therefore, although intervention was not the focus of the current thesis, the purpose of this thesis was to take a multi-disciplinary approach in identifying key role players of resilience and risk in a South African adolescent population, and by doing so, and with reproducible data generated by other studies, future intervention strategies can be adapted or tailored accordingly. Consequently, we simultaneously assessed the respective associations of trauma exposure and anxiety proneness with measures affording either resistance or vulnerability to pathology, and correlated these largely psychological and neurophysiological measures with peripheral indicators of stress sensitivity and immune status, so as to progress towards a precision medicine paradigm.  By elucidating major role players and links between the brain and body, we aimed to highlight specific avenues to target with holistic intervention strategies.

In order to orientate the reader, we have provided an overview of the relevant literature on the topics introduced above in the next section, followed by the statement of our hypothesis and main aims. A detailed account of the experimental work that was conducted follows after that.

# CHAPTER 2

# OVERVIEW OF THE LITERATURE

Given the multi-disciplinary nature of our approach, several aspects have to be considered in terms of the available literature. In order to facilitate clarity of the argument, this chapter is presented in two main divisions. Part A provides an overview of the psycho-neurophysiological underpinnings of anxiety, on the backdrop of childhood trauma. The focus is therefore firstly on the broader psychological characterisation of individuals in terms of anxiety, as well as the known potential modifiers, for example, trauma exposure. Given that adolescents are the population of interest for the purpose of this thesis, we then provide a more in-depth analysis of factors which may increase risk for development of anxiety in children. In addition, the literature on information processing is reviewed in terms of pathology in the context of anxiety and/or trauma. In Part B, a comprehensive review of available data on neuroendocrine adaptation and maladaptation to trauma and anxiety is provided, with specific focus on the hypothalamic-pituitary-adrenal (HPA)-axis and its links to the immune system in this context. Both sections end with a brief discussion of potential targets for intervention.

**Part A: Psycho-neurobiological underpinnings of anxiety in relation to childhood trauma**

**2.1 Anxiety: Nature following nurture**

In addition to external provocations rooted in the environment, such as traumatic events and early life experiences with caretakers, the manifestation of anxiety is also dependent on personality dimensions and the modulating physiological make-up of the individual. These latter factors include neural, endocrine and immunological underpinnings of either vulnerability or resilience to the development of anxiety.  Epigenetic studies in humans have indeed recently revealed that fewer genes are being actively transcribed in individuals who have suffered abusive childhoods, with most of the genes that were hyper-methylated (and thus not expressed)  related to neural plasticity (Labonte, et al., 2012; Naumova, et al., 2012). This observation has led to the proposition that poor parental care may result in fewer plasticity-related genes being expressed, resulting in potential inhibition to learn, remember or adapt within the environment - thus rendering the individual less resilient (Peckham, 2013). This, in turn, alludes to the notion that the genes involved in the aetiology of anxiety, and its manifestation throughout life, are shaped by experience, and, in this sense, that there is an influence of nurture on nature which allows for the expression of genes to be continuously modified, depending on changes in the environment and the degree of plasticity of the genome. In this way, broadly speaking, one's environment,

especially in early childhood, dictates the formation of, for example, specific personality dimensions (see 2.2.1) and attachment styles (see 2.2.2), as well as the stability and/or evolution thereof over time. Therefore, before attempts can be made to identify markers of therapeutic targets to achieve/improve resilience, a closer look at the psychological aetiology of anxiety is required.

### 2.1.1 Defining anxiety

Anxiety disorders are among the most prevalent of psychiatric disorders across all age groups, as confirmed by several epidemiological studies (Adewuya, et al., 2007; Beesdo, et al., 2009; Costello, et al., 2006; Roberts, et al., 2006). The onset of an anxiety disorder is typically in childhood or early adolescence (Kessler, et al., 1994). The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) identifies 12 primary anxiety disorders (DSM-5, 2013), which testifies to the complexity and diversity of the conditions collectively termed anxiety, although many common characteristic symptoms also present across the spectrum of anxiety disorders (Brantley, 2003).

In the context of neurophysiology, some theorists have presented a definition of anxiety as a blend of various emotions and cognitions or memory stored in affective networks (Cloninger, 1986; Gray, 1982). However, anxiety is context- and stimulus-specific, and whether it is expressed as pathological or adaptive, depends on the activation state of the various brain regions employed, which in turn depends on the particular classification of stimuli into combinations of four functional categories (actual, potential, avoidable and unavoidable) (Gray & McNaughton, 2000; Middeldorp, et al., 2005). Aside from the stimulus-specific view of anxiety, Barlow has conceptualized anxiety as "a loose cognitive-affective structure which is composed primarily of high negative affect, a sense of uncontrollability based on future threat, and a shift in attention to a primarily self-focus or a state of self-preoccupation" (Barlow, 1991). This condition, accompanied by chronic hyper-arousal and vigilance, is one whereby threat is anticipated in the future and is interpreted as frequently changing (Fridlund, et al., 1986). The looming vulnerability aspect of anxiety pertains to the anticipatory state of preparation to deal with threat (Wheeler, et al., 1997) and has been found to be a stable trait and unique to the construct of anxiety (Riskind, et al., 2000).

Accordingly, anxiety is then rooted in both nature and nurture, whereby context-specific nurture (or rather the lack thereof), in the form of a history of childhood maltreatment, has been consistently linked to increased risk for development of an anxiety disorder (Afifi, et al., 2009; Arata, et al., 2007; Ethier, et al., 2004; Springer, et al., 2007). This finding, in turn, has been associated with the anxiety proneness denoting nature (Handa, et al.,

2008; Scher & Stein, 2003), or more specifically the personality dimensions of anxiety sensitivity (Leen-Feldner, et al., 2008) and trait anxiety (Hensley & Varela, 2008). Accordingly, trait anxiety and anxiety sensitivity (AS) have been associated with the development of anxiety disorders in general and, based on heritability studies, the notion of trait underlies both attributes (Legrand, et al., 1999; Stein, et al., 1999). AS is a dispositional characteristic and a measure of an individual's beliefs that anxiety-related sensations and symptoms pose physical, psychological and/or social risks (Reiss, 1991; Reiss & McNally, 1985). In contrast, trait anxiety refers to the tendency to respond to all stressors in a fearful manner (McNally, 1989).  Measures of AS have been moderately correlated with trait anxiety, resulting is the recommendation that indexes of trait anxiety be included in investigations focusing on relationships  between AS and other variables, given that at least some of the findings attributed to AS might in fact be accounted for by trait anxiety (Lilienfeld, et al., 1989; Lilienfeld, et al., 1993).The term 'anxiety prone' has therefore been adopted to refer to individuals scoring high on both accounts of trait anxiety and AS (Stein, et al., 2007).

Considering both nature and nurture, we propose a cyclic model based on the concept of controllability (Fig. 2.1), by which anxiety-prone individuals are characterised by motivational systems underlying the impulsion to control the external environment (situations, people, or events), either to attain comfort or avoid discomfort. However, since it is impossible to control every aspect of one's life, inevitable conscious or unconscious feelings of helplessness and lack of control ensue. This is in response to real or imagined fear-based stimuli being interpreted as uncontrollable.  More specifically, this detection and labelling of threat as uncontrollable leads to negative affective states, subsumed by fear and hyper-arousal, which, in turn,  leads to cognitive and behavioural passive avoidance, as well as emotional regulation aimed at regaining control by avoiding dealing with the sense of uncontrollability. This scenario is based on neurocognitive evidence supporting the avoidance hypothesis, which states that anxious individuals tend to inhibit or avoid deep processing of threatening information leading to cognitive avoidance of threatening stimuli during later stages of information processing (Mansell, et al., 1999; Mogg, et al., 2004; Sposari & Rapee, 2007).

This model suggests that anxious individuals are biased to the selection of threatening stimuli, and to interpretation of stimuli, in general, as negative, as well as prone to the acquisition and triggering of conditioned fear, with these processes taking place in subcortical brain regions during the initial stages of stimuli processing. However, this information is avoided in later stages of information processing, where emotion regulation goals underlie attentional avoidance *via* cognitive reappraisal. Down-regulation of emotion through cognitive

reappraisal has been found to uniquely recruit regions in the dorsolateral prefrontal cortex (DLPFC) (Ochsner, et al., 2004), as well as the ventrolateral prefrontal cortex (VLPFC), where the latter region has been correlated with decreased activation in the amygdala and reduced negative emotional experience during cognitive reappraisal, suggesting that the VLPFC may be associated with conscious regulation of emotional processes (Wager, et al., 2008). Therefore, increased VLPFC and DLPC function may be associated with increased use of regulatory strategies, such as cognitive avoidance and behavioural inhibition, in response to anxious hyper-arousal (Hofmann, et al., 2012). However, this takes place potentially at the expense of other more adaptive regulatory processes such as (conditioned fear) extinction learning (Phelps, et al., 2004; Schienle, et al., 2007), and provides only temporary relief to negative affect associated with anxiety. Thus, selecting coping strategies such as cognitive and behavioural avoidance with concomitant affect regulation, signifies failed attempts at regaining control, and therefore serves as input in information processing, in the form of fear-based stimuli, with subsequent motivation to control external factors. And so the cycle continues. From this point of view, it is evident that it is not so much the external factors, but how these factors are being perceived, that causes anxiety.

**Figure 2.1.** Proposed model underlying the perpetuation of anxiety
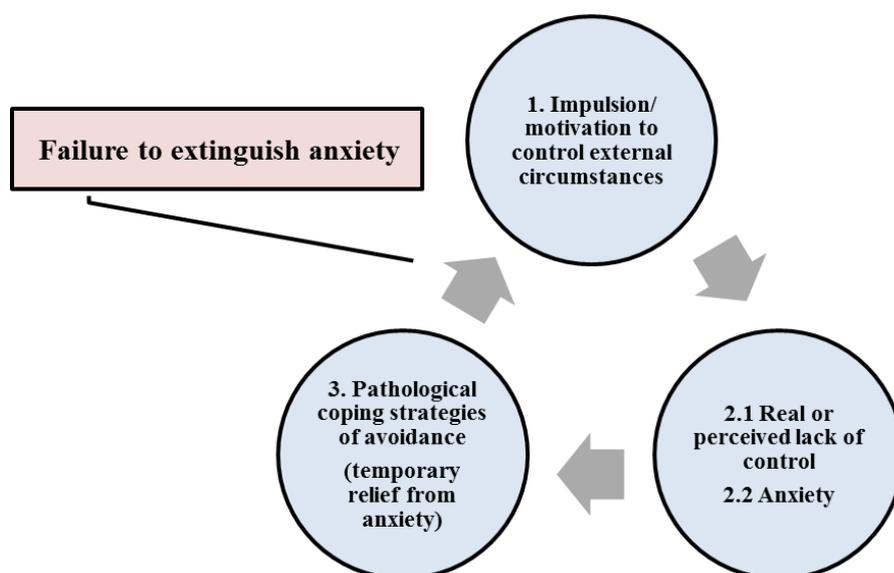


Having defined anxiety, the next few sections will review the diagnosis of anxiety, as well as potential developmental and other factors which come into play during adolescence, and which may lead to the development of anxiety by affecting psychological or physiological systems.

**2.1.2 Diagnosis of anxiety in the context of trauma**

In order to administer precise, tailor-made treatment(s) according to a patient's unique physiological and psychological makeup, the diagnosis and characterisation of the exact nature of a patient's condition is vital. The two long-standing versions of diagnostic systems for psychiatry, the DSM and the Mental and Behavioural Disorders section of the International Classification of Diseases (ICD-11), have shown limitations regarding the nosology of anxiety disorders in the context of neurophysiology. Furthermore, specifically in the context of anxiety in children, these systems have proved inadequate as tools to distinguish anxiety-disordered children from children without psychiatric diagnoses, when considering that anxiety symptoms are experienced as part of normal developmental growth and maturation (Schniering, et al., 2000) and thus lie on a continuum (Sanislow, et al., 2010). Furthermore, symptoms experienced by children are evaluated by the DSM on the basis that children are aware of their feelings and are able to appropriately label and communicate these (Kirkpatrick & Davis, 1994). This assumption is flawed however, as it has been shown that only after the ages of about 8 to 9 years, are children able to provide accurate reports of their emotions (Stone & Lemanek, 1990), while the capacity for true introspection is only fully developed during adolescence (Harter, 1990).

Addressing these issues, a new approach in psychiatric diagnosis is emerging, that of assessing varying degrees of pathophysiology, i.e. disruptions of normal range functioning as downstream products of psychopathology. In fact, the National Institute of Mental Health (NIMH) instituted the Research Domain Criteria (RDoC) project in early 2009, in an attempt to develop new ways of classifying mental disorders that is based in part on neurobiological research aimed at promoting individualized treatment, now dubbed 'precision medicine' (Cuthbert & Insel, 2013).

Despite these relatively recent efforts, a comprehensive model of the continuity and change in anxious emotion, along with the accompanying biology in childhood anxiety, does not exist. As a general guideline, it has been theorised that separation anxiety symptoms and primal fears is evident around ages 6–9 years, followed by generalized anxiety symptoms and fears concerning danger and death around ages 10–13 years. Social anxiety symptoms and social performance associated fears only become evident in adolescents around age 14–17 years (Warren & Sroufe, 2004; Weems, et al., 1998). It has been theorized that these normative age specific characterization of anxiety symptoms are secondary to and shaped by core features such as trait anxiety, with trait anxiety considered a predisposition to experiencing anxiety sensations (Spielberger, 1972), as well as dictating the course of biological processes (Puliafico & Kendall, 2006; Weems & Stickle, 2005). This

highlights the need for a model identifying specific processes that underlie continuity and change across trajectories of the core features, including specifically neurophysiological determinants of anxiety-disordered emotion, which then, in turn, could modify the specific expression and trajectories of the secondary features. However, in determining causes and categories of psychopathology in biological mechanisms, conceptualizing disorders in an essentialist way that oversimplifies reality should be guarded against (Nesse & Stein, 2012). Nevertheless, such a model should ideally encompass the neurophysiology associated with the core feature of trait anxiety, as a trajectory across childhood. Furthermore, such a model would account for the modern age child growing ever more precocious, especially in the third world domain, with guidelines of age-specific anxiety symptoms as well as identification of specific subscales of childhood maltreatment that pose greater risk for psychophysiological maladaptations. In order to investigate the proposed model, we have measured trait anxiety and anxiety sensitivity as core features modulating neurophysiological outcomes and mediating the secondary feature of self-esteem (a pertinent vulnerability/resilience factor in the experience of anxiety, specifically associated with our study population's age group). We have also measured levels of childhood trauma and subscales so as to investigate interactions of trauma with the aforementioned secondary and core features of anxiety as well as the relative contributions of anxiety *versus* trauma exposure in neurophysiological maladaptations.

Turning to diagnosis, or quantification of the extent of trauma exposure, childhood maltreatment is characterised as acts of commission or omission by parents or caregivers that result in potential harm to the child's health, and include experiences such as physical, sexual and psychological abuse, as well as physical or emotional neglect (Daruy-Filho, et al., 2011). Cook and her group have highlighted a theoretical framework encompassing multi-faceted, child-caregiver based "complex trauma" in children and adolescents, which includes emotional abuse, physical neglect, sexual and physical abuse as well as witnessing domestic violence, ethnic cleansing, and war as forms of maltreatment/trauma (Cook, et al., 2005). This take on maltreatment seeks to remedy limitations of classical DSM classification of disorders which does not fully capture a traumatized child's complex self-regulatory and relational impairments. Recently, childhood abuse and neglect, specifically, have been conceptualized as "toxic" stressors resulting in strong, frequent, or prolonged activation of the body's stress management system and which are experienced with the absence of caring adults lending support, contributing to adult chronic disease (Jaffee & Christian, 2014). However, we suggest that an assessment of childhood abuse and neglect be used in conjunction with anxiety measures. The relative contributions of anxiety and trauma to

clinical outcome have not been elucidated in a population with a high incidence of complex trauma but with no current diagnosis of post traumatic stress disorder (PTSD), such as in the case of our study population.

**2.2 Childhood risk factors for the development of clinical anxiety**

**2.2.1 Personality dimensions governing neurophysiological attributes**

One of the first psychobiological models of personality identifies two basic dimensions of personality, i.e. extraversion and emotionality, associated with differences in nervous system functioning (Eysenck, 1967). A later three-dimensional theory of temperament and character, constitutes three broad heritable personality dimensions (Cloninger, 1986), each exhibiting a separate neurobiological basis: a) novelty seeking that has been correlated with low basal dopaminergic activity, b) harm avoidance (anxiety proneness) which correlates with coexisting high serotonergic activity (physiological hyper-arousal), and c) reward dependence which correlates with low basal noradrenergic activity. Advances in research on brain function has allowed for more refined views on personality to be developed, specifically in terms of anxiety proneness and impulsivity as dimensions of personality, building on the previously formulated Reinforcement Sensitivity Theory (RST) of Gray (Gray, 1982).

These two dimensions are higher order traits that represent distinct central nervous system structures as they can be separated both pharmacologically and physiologically by brain lesions (Gray, 1987a; Gray, 1987b; Quay, 1993). The personality dimension of anxiety proneness modulates the aversive motivational system called the behavioural inhibition system (BIS), comprising the septohippocampal system and amygdala, with afferents from the brainstem to the frontal lobe (Gray, 1982). According to Gray, the BIS represents goal conflicts and is responsible for the experience of negative affect such as fear, anxiety, frustration, and sadness in response to cues of punishment, non-reward and novelty, with consequent inhibition of behaviour that may result in negative experience (Gray, 1987b). The BIS is activated during early years as it has been shown that inhibited children tend to have either abnormally high (Kagan, et al., 1987) or abnormally low (Fox & Stifter, 1989) basal heart rates and other markers of sympathetic arousal and a lowered threshold or response (possibly in the limbic system) to novel situations, which in turn leads to behavioural inhibition due to sympathetic system arousal and subsequent discomfort (Beauchaine, 2001; Kagan, et al., 1990; Manassis & Bradley, 1994). Activation of the BIS also results in endocrinological responses, such as increased cortisol release (Ryan, 1998), and hypo-activity of the HPA axis may be an indication that the BIS is deficient (McBurnett, et al., 1996).

Conversely, the dimension of impulsivity controls appetitive motivation and has been termed the behavioural activation/approach system (BAS) (Fowles, 1980; Gray, 1987a), residing within networks of catecholaminergic and, especially, dopaminergic pathways, although these are less defined than the neural basis of the BIS (Stellar & Stellar, 1985). Signals of reward, non-punishment, and escape from punishment activate the BAS with increased movement toward goals and the experience of positive affect such as hope, elation, and happiness (Depue & Iacono, 1989; Gray, 1990). Individual differences in affect and behaviour to a given stimulus then reflect differences in combinations of BIS and BAS sensitivity. The current conceptualization of RST (Corr & McNaughton, 2008) posits an additional third neurobiological system, the fight–flight–freeze system (FFFS), which involves the amygdala and the hypothalamus (a key component of the HPA-axis).  The FFFS is based on defensive-avoidance behaviour, is activated in response to aversive stimuli, promoting escape, avoidance, or confrontational behaviours associated with fear and panic. It is activation of the BIS which is thought to be responsible for increased risk in developing an anxiety disorder (Wilt, et al., 2011), and research supports the use of the BIS scale as a combined measure of BIS and FFFS sensitivity (Pickett, et al., 2012; Smillie, et al., 2006), with the term ''BIS sensitivity'' denoting a combined measurement of BIS and FFFS sensitivity. However, according to Rosalsky ((Rosalky, 2013)), the FFFS and the BIS are distinct systems of fear and anxiety, respectively, and their group provides evidence for increased cortisol release associated with FFFSs activation and decreased cortisol release with BIS activation. These findings are in contrast to outcomes reflecting hyper-activation of the HPA-axis associated with the BIS, reported by the aforementioned authors (McBurnett, et al., 1996; Ryan, 1998). Nevertheless, we suggest that activation of the FFFS denotes a more acute activation of the HPA-axis in response to fleeing fear-eliciting conditions, whereas the BIS is associated with a more diffuse and chronic condition of anxiety. Therefore, under conditions of predominant BIS activation, the HPA-axis has adapted to raised cortisol levels associated with frequent FFFS activation, by decreasing cortisol output *via* negative feedback at the level of the pituitary.

Thus, within the domain of psychobiology, cognitions, desires (motivation), behaviour (approach/avoidance) and affect (positive/negative) underlie responses to conditioned cues of punishment and reward, suggesting that the RST is a good candidate for an all-encompassing model of personality. However, one concern denoted in the literature is that BIS sensitivity is measured by trait anxiety scales, which do not allow for a distinction between vulnerability to anxiety for a given level of threat and frequency of anxiety over time (Carver & White, 1994; Fowles, 1987). In other words, an uncoupling of affect (anxiety) and behaviour needs to be established when determining the strength of the BIS *versus* the BAS. Another issue to be aware of is that Gray's motivational

model considers responses to conditioned stimuli (Fowles, 1987). Sensitivity to unconditioned punishment may therefore influence a person's level of state anxiety, whereas BIS sensitivity to conditioned punishment would reflect levels of trait anxiety.

In context of the current study, we focused on the personality dimensions of trait anxiety, anxiety sensitivity, as well as self-esteem, as functions of risk/resilience in the manifestation and/or progression of neurophysiological maladaptation. This is based on the view that personality constitutes a complex organization of systematically interrelated trait dispositions (Watson, et al., 1994), where specific personality traits can predispose an individual to develop an anxiety disorder, especially in conjunction with a relevant stressor serving to trigger the onset of the disorder (diathesis-stress model), with the course of the disorder, in turn, influencing those traits. This alludes to the fact that the anxiety disorders reflect an underlying hierarchical organization. They all share a common higher order dimension of neuroticism/negative affect, which represents a general vulnerability to stress. Specific phenotypes of anxiety contain unique lower order traits (where each lower order trait, in turn, can be subdivided into multiple, even lower order dimensions).

For example, it has been proposed that the higher-order dimension of Neuroticism consists of at least the following lower-order traits: anxiety, depression, low self-esteem, tenseness, shyness and moodiness (Eysenck, 1997). Therefore, when conducting research on the aetiology of anxiety, a study of the lower-order traits can provide more detailed information on specific phenotypes of anxiety (Livesley, et al., 1998). Accordingly, Zinbarg and colleagues have provided evidence for a three-level hierarchy of personality dimensions with different combinations giving rise to various types of anxiety disorders: a) a single, higher order, general factor; (b) several lower order level factors indicating discriminability among the key features of the anxiety disorders; and (c) factor-analytically derived subscales that represent even narrower constructs at the lowest level (Zinbarg & Barlow, 1996).

Thus, relevant to the current study, we decided to include two higher order measures of negative affectivity, i.e., AS and trait anxiety, constituting general factors of anxiety, and, in addition, we have assessed more specific vulnerabilities giving rise to these features, which may potentially serve as markers of risk/resilience in the event of experiencing ongoing trauma. These lower order dimensions include measures of handedness and self-esteem. In light of individual differences in personality as a function of degree of handedness, it has been found that consistent-handers (high degree of consistency with which the preferred hand is used over the non-preferred hand) are less sensation seeking, more authoritarian, and more sensitive to disgust (Christman, 2014). A more

comprehensive review of handedness follows in section 2.4.4.2. Specific to the study of self-esteem and personality in adolescents, self-esteem can be measured as a personality dimension by means of the Rosenberg Self-esteem Inventory. Temperamental dimensions contribute to how well individuals adapt to the challenges of adolescence, which, in turn, contributes to self-concept development (Klein, 1992; Windle & Lerner, 1986). This view incorporates both a genetic component (Kendler, et al., 1998; McGuire, et al., 1999; Neiss, et al., 2009), as well as a social component (Harter, 2006) of self-esteem, in that genetically influenced individual differences are associated with how individuals are perceived by important figures in their lives and with how individuals perceive their social and physical environments (Caspi & Shiner, 2008).

**2.2.2 Childhood maltreatment in the context of the maternal care model**

Attachment originates in early childhood and serves as a child's biological adaptive system whereby covert internal working models of self and others become engrained. Infants, with their still developing neurophysiological systems, are dependent on a caretaker for survival.  This time period, as well as the following few years of childhood, signify a window of vulnerability to the detrimental effects of insecure attachment which, in extreme cases, is due to trauma experienced as a result of maltreatment by significant others.

Poor maternal care has been shown to alter glucocorticoid sensitivity in rats in the well-known "lick and groom" studies, where rat pups of uncaring mothers were more prone to develop anxiety than sibling pups placed with caring mothers immediately after birth (Weaver, et al., 2004). More recently, this data was expanded upon. Research in epigenetics and environmental programming, focused on causal relationships between maternal behaviour and gene expression in rodent models, have illustrated that the regulating effects of maternal care on glucocorticoid receptor (GR) levels, previously described, were the results of epigenetic modification of hippocampal GR exon $1_7$ (Fish, et al., 2004; Meany & Szyf, 2005; Sapolsky, 2004).  It seems feasible then that, while genes provide the blueprint for the organization of the brain and for triggering the onset of developmental phases, experience serves to shape ongoing gene expression responsible for the development of neural and/or endocrine trajectories. It is not surprising then that functional, perfusion, and structural magnetic resonance-based imaging has provided evidence suggesting that life stressors may activate specific epigenetic effects in, for example, the serotonin transporter gene, which in turn has been associated with amygdala and hippocampal resting activation, as well as functional connectivity between the amygdala and hippocampus and alterations in grey matter structural features (Canli, et al., 2006).

However, in terms of trauma, the aforementioned research in the preceding two paragraphs has been based on an animal maternal nurture model as well as life stress in humans, in general. Trauma experienced as a result of childhood maltreatment is more complex however, and may be associated with attachment schemas. Scholars in attachment theory have identified four distinct patterns or styles of attachment, namely secure, insecure-avoidant, insecure-ambivalent and disorganised, with the different types of insecure attachment suggested to manifest in different types of anxiety disorders during childhood (Manassis & Bradley, 1994). For example, it has been shown that children with avoidant and disorganised attachment schema have potentiated levels of biological markers of sustained stress and trauma (Spangler & Grossmann, 1993) and these insecure attachment patterns correlate with child abuse (Beeghly & Cicchetti, 1994).  Although attachment styles are established in early childhood, these internal working models serve as conscious or unconscious relationship templates right through into adulthood where romantic partners adopt the role of primary caregivers (Ainsworth, 1969). These models are not easy to modify or replace, as early attachment styles are often regarded as strong predictors of either positive adaptations or maladjustments later in life (Zimmermann, 2004). Further research is required to determine the chronic modulating effects of attachment schemas on physiology, given the fact that specific attachment schemas have been shown to persist into adulthood (Bartholomew & Horowitz, 1991). However, contrary to the view of an inherently static and cemented attachment style, some cases have been documented whereby individuals have actively overcome hazardous parenting circumstances and have not fallen victim to attachment insecurity later in life (Pearson, et al., 1994; Roisman, et al., 2002), indicating a promising avenue for modulation *via* intervention. Given the fact that literature on attachment styles is still incomplete, this factor was not considered as a predictor of outcome in the current thesis. However, it is important to keep in mind this potential confounder when interpreting data.

**2.3 Information processing**

Stimuli are organised within neural networks in the form of memories, self-referential networks and attachment schemas which are based on the nature of relationships with primary caregivers during early childhood. Despite the individual cases mentioned above, for which more positive therapeutic outcomes have been reported in the context of attachment styles, the literature seems to agree that  ongoing trauma experienced in early childhood (as opposed to an acute traumatic event in adulthood) significantly modifies the architecture of neural circuits. These circuits have been associated with the mediation of cognitive-emotional regulation, vigilance, arousal, and the integration of endocrine, autonomic, and immune regulatory systems in ways such that certain patterns

of connectivity become highly stable and, therefore, the neural paths of least resistance. (Ladd, et al., 2000; Meany & Szyf, 2005). However, the literature to date has not differentiated anxiety as a co-factor and therefore it still remains unclear as to whether anxiety proneness or trauma exposure is the major contributor to the above mentioned (mal)adaptations observed. Below, we have briefly described stimuli processing scenarios, which can be either adaptive or maladaptive, the latter with undesired outcome.

**2.3.1 Adaptive stimulus processing: Where psychology meets physiology**

During ordinary stimulus processing, at any given moment in time, stimuli are presented to the central nervous system *via* 1) the five senses, 2) mental imagery, and 3) signals from the periphery relaying perturbations in the sympathetic-, endocrine, and immune systems by means of bodily sensations and blood borne chemical messengers crossing the blood brain barrier. All stimuli are represented within frameworks of past, present, or future, so as to construct the notion of an independent (separate from other), enduring self or identity and is continuously being generated in the context of a spatial experience (D'Argembeau, 2013; Klatzky, 1998).

Stimuli reaching the brain or originating within the brain, are organised within a tri-modal orientation of the self - namely, egocentric, allocentric, or ecosystematic/existential (Brentano, 1973) - and without self-awareness, the usual sense of self is egocentric, creating subject-object duality (me and you) (Purser, 2012). Adopting an egocentric stance is adaptive only in the absence of stressful scenarios, whereas processing of stimuli in the allocentric mode allows for the self and others to be observed by means of meta-cognitive awareness or mindfulness, permitting more flexibility in action under all conditions (Teasdale, et al., 2002). Inasmuch the mitigating of anxiety is concerned, the non-dualistic existential orientation is the most ideal schema to be employed, as it allows for awareness of unconscious material (Cloninger, 2006) and results in "the capacity to live fully in the present, and respond freely and flexibly to new experience without fear" (Deshmukh, 2008).

Further distinctions of stimuli processing within the tri-modal orientation can be made based on the level of consciousness attained: the unconscious egocentric mode arises in brain circuits during infancy, where discomforting bodily symptoms are conditioned with self and reflect a direct threat to the continuation of the self, whereas with developing a degree of self-awareness, the more conscious allocentric and/or existential modes are employed, with bodily symptoms viewed as processes which are impermanent and unattached to the concept of "me/mine" (Austin, 2011). Therefore, a distinction can be made between processing of stimuli of unconscious nature, and therefore occurring automatically, *versus* those that reach the level of conscious awareness through the process of, among others, executive monitoring (Pribram, 1999). These are based on two

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major neural circuits of stimuli processing: The first is the reflexive, unconscious, *fast system* which relays information immediately from the sense organs through the thalamus to the amygdala, and from there to the hypothalamus. Simultaneously, the *slow system* sends information from the thalamus first to the cortex and hippocampus, and only once it has been processed in these regions is the signal projected to the amygdala and hypothalamus. This system is slower as it contains more synaptic connections and involves conscious processing. This system also tends to inhibit downstream peripheral responses as it involves processing in the cortex (LeDoux, 1986).

Regardless of whether the fast, or slow, or both systems are activated, this information reaches the hypothalamus. From there the hypothalamic-pituitary-adrenal (HPA)-axis and the sympatho-adrenal medullary (SAM) pathway (also called the Locus Ceruleus Norepinephrine (LC/NE) sympathetic system) emanate. The hypothalamus can therefore be viewed as the region of interface between the brain and the body. The duration, intensity, frequency and type of stimuli being processed all influence the degree of activation of both the HPA and SAM systems. Pathways converging at the paraventricular nucleus (PVN) of the hypothalamus causes corticotropin releasing hormone (CRH) to be released, which then travels from the median eminence down the hypopheseal portal vessel to the anterior pituitary where ACTH is released into circulation. ACTH stimulates the adrenals to release cortisol which, in turn, feeds back in a negative fashion to the pituitary and hypothalamus, in addition to exerting a suppressive effect on the immune system (Segerstrom & Miller, 2004). The HPA axis and the SAM  pathway constitute a positive reverberative loop, in that activation of one system leads to activation of the other by means of cross-talk between the LC and CRH-secreting neurons in the lateral PVN (Elenkov, et al., 2000).

Stimulation of the LC by projections from the PVN results in NE being released throughout the brain. The signal also travels from the LC in the brain stem to preganglionic nerves in the spinal cord, which ultimately causes the release of NE from the varicose sympathetic nerve terminals and epinephrine from the adrenal medulla. The 10th cranial nerve, also called the vagus (originating in the medulla and leaving the CNS at the neck to innervate the viscera), provides the CNS with sensory information about the state of the body's organs and facilitates parasympathetic tone. An increase in sympathetic drive *via* the SAM pathway results in an increase in vagal tone, which serves to modulate the parasympathetic nervous system during the experience of psychosocial stress (Raison, et al., 2006; Sahar, et al., 2001; Wright, et al., 1998). More specifically, baseline

levels of cardiac vagal tone and vagal tone reactivity (which can be accurately tested and quantified) have been associated with the expression of emotion, and self-regulation skills (Porges, et al., 1994).

When no more stress signals reach the hypothalamus, HPA axis and SAM pathway activation are down-regulated and the body's physiology returns to its basal state. However, during conditions of chronic activation of the HPA-axis and SAM pathway due to childhood trauma, we suggest a condition dominated by maladaptive stimuli processing, which is compounded by the presence of anxiety proneness functioning as an enduring trait.

**2.3.2 Maladaptive stimulus processing in the context of anxiety and trauma**

Traumatic experiences during childhood are engrained in both conscious and unconscious networks *via* long term potentiation or Hebbian learning (LTP) (Hebb, 1949) so that during subsequent years, the environmental stimuli are processed within these conditioned pathways in the brain, with bidirectional communication between the brain and the body. Even after the source(s) of the trauma has been removed, repressed memories and emotions can resurface when collections of certain stimuli resemble that of the initial trauma, with these pathways  activated within a timeframe preceding that of conscious control and awareness (Brewin, 2006; Ferster, 1973; Greenberg & Mitchell, 1983). Accordingly, on a neurological level, evidence is provided for the simulation hypothesis where thinking consists of simulated interaction with the environment, i.e. simulation of behaviour, perception and anticipation (Hesslow, 2002). In addition, with maladaptive stimuli processing, there seems to be a selection bias for stimuli signalling potential danger (Burgess, et al., 1981; MacLeod & Hagan, 1992; Mogg, et al., 1989). Indeed, in adults, heightened anxiety, as measured by the Spielberger's state-trait anxiety scale, was correlated with increased perception of angry and fearful faces, along with decreased perception of happy expressions, in effect providing evidence of involuntary selection of threat in anxiety (Gray, et al., 2009). A similar study on paediatrics with different anxiety disorders provided evidence for anxiety disorders, in general, to be associated with an attention bias towards threat (Roy, et al., 2008). This sensitivity towards selecting fearful stimuli is also evident in individuals scoring high on AS, as there exists a tendency to respond fearfully to anxiety-related bodily sensations and symptoms, and these fears are thought to intensify pre-existing anxiety (Reiss, 1991).

Furthermore, adding complexity to the problem, anxiety-provoking stimuli do not have to be real in order to elicit neurophysiological responses.  It has been postulated that individuals prone to experiencing anxiety will continuously mentally simulate hypothetical outcomes to events that involve rapidly rising risk in the form of harm or threat (Hsee & Abelson, 1990; Taylor & Pham, 1996). Activation of neural networks derived from

environmental, physiological/somatic and mental stimuli culminate at the level of the thalamus, with the net sum of afferent projections to the thalamus activating downstream peripheral stress pathways *via* the SAM pathway and HPA axis. However, the degree to which these two stress pathways are tonically activated and responsive to stressors depends on whether adaptive or maladaptive stimuli processing in higher order brain regions takes place.

Maladaptive processing is thought to occur within an egocentric framework (refer to section 2.3.1) and has been implicated in anxiety disorders such as social phobia, where subjects become exceedingly self-focused and distort interpretations of events that are highly self-referential to that of actual threats to survival (Clark & Wells, 1995; D'Argembeau, et al., 2006). Under these conditions, subjects see themselves from an external point of view, characterised by decreased activity in regions of the cerebral cortex associated with interoceptive awareness (Eich, et al., 2009). Organization of stimuli in an egocentric framework in this context are thought to be a paradoxical defence against the experience of anxiety as it creates the illusion of control, autonomy and an ontological sense of self (Purser, 2012). In addition to the maladaptive egocentric processing, there is also a shift towards fast system processing at the expense of the slow system (Liotti, et al., 2000; Tromp, et al., 2012). Strong support has been provided for a common amygdala–prefrontal circuitry characteristic of anxiety, which underlies selective attention to threat, negative (or less positive) interpretations of emotionally ambiguous stimuli, and more acquisition with less extinction of conditioned fear (for a review, see (Bishop, 2007)).

Figure 2.2 provides a summary of maladaptive stimuli processing in the context of anxiety, on the backdrop of a history of childhood maltreatment and insecure attachment. In order to incorporate our model of uncontrollability and the construct of anxiety, figure 2.1 is superimposed.  Interactionism exists between situational cues, and anxiety associated temperamental traits and dispositions such as trait anxiety (Legrand, et al., 1999) and AS (Stein, et al., 1999) which, in turn, influences physiological sequelae and contribute to the experience of anxiety symptoms as well as cognitive, emotional and behavioural inhibition. A more detailed description of this model involves individuals characterised by high trait anxiety, AS, and BIS activation and low levels of trait self-esteem. These traits facilitate the selective attention to anxiety-provoking sources of stimuli stemming from 1) the environment, in the form of conditioned trauma-associated cues, 2) catastrophizing (mental simulation of fear-based outcomes), and 3) the periphery, *via* bodily sensations and chemical messengers influencing mood/affect. Pathological processing of stimuli arising from these sources underlie the motivation to control these and resides within egocentric networks, predominantly fast system

processing, insecure attachment schemas, as well as pathological coping networks of cognitive and emotional avoidance. Activation of these neural pathways results in stimuli being labelled as negative and uncontrollable, and creates a sense of an ontological enduring self. Furthermore, pathological information processing also results in perturbations in the immune- and endocrine systems. In this way, a cycle consisting of the motivation to control and preserve a sense of self, and subsequent feelings of loss of control, persists, based on incongruence between self-referential feedback and external situations. This ultimately manifests in anxiety symptoms and maladaptive behaviour aimed at either avoiding pain or gravitating towards pleasure.

**Figure 2.2.** Anxiety associated information processing model: pathological stimuli processing under conditions of chronic childhood maltreatment
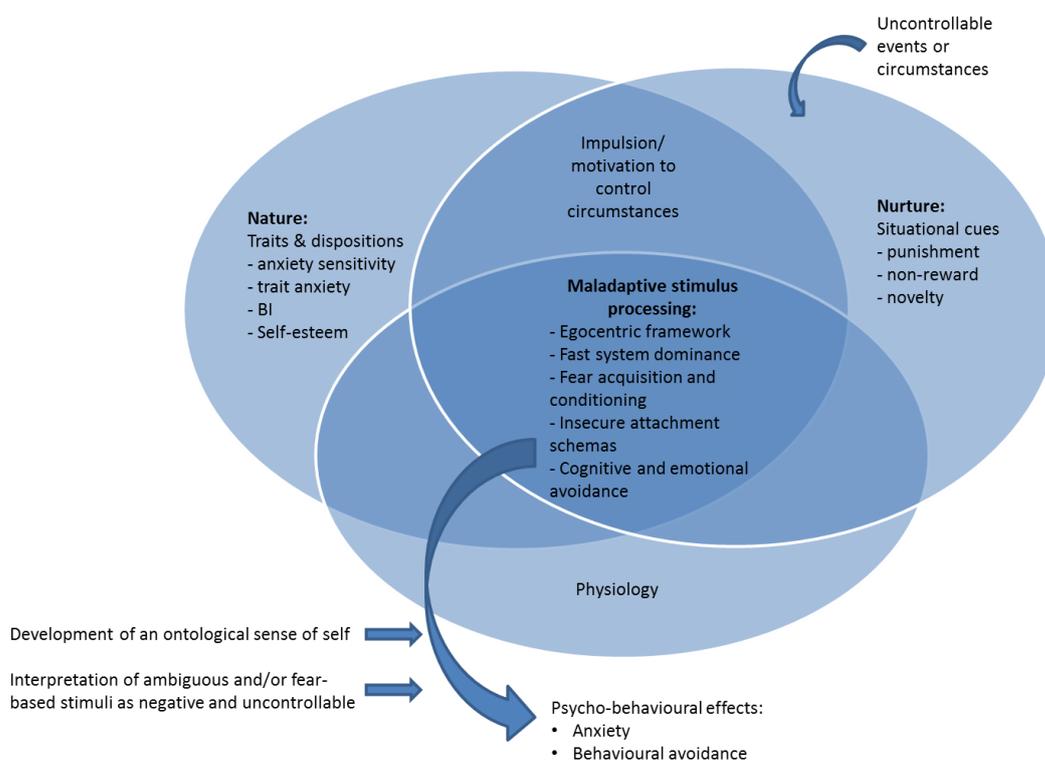


From this model, it can be deduced that the most ideal target for intervention would be the area where all three circles overlap, as it addresses all three aspects of nature, nurture and physiology. These could take on the form of therapy and (ideally) preventative psychological intervention. When considering the individual circles separately - seeing that it is nearly impossible to shield children from experiencing trauma and from undesired situational cues, especially in low socio-economic communities, and seeing that temperamental traits are for the most part engrained - next in line of optimal intervention choices are those that are physiologically based: either using physiological systems as a diagnostic/monitoring tool, or managing these by means of medication.

**2.4 A role for neurophysiology?**

In the context of childhood maltreatment, it is becoming increasingly evident that a harsh family climate manifests in one's neurophysiological make-up (Loi, et al., 2014), resulting in vulnerability to diseases such as autoimmune diseases that arise decades later (Miller, et al., 2009a). As previously mentioned in the section on attachment styles, in the context of epigenetics, physiological mechanisms leading to this chronic maladaptive state includes a higher percentage GR gene methylation (van der Knaap, et al., 2014) and thus epigenetically modified down-regulation of GR expression in the hippocampus, resulting in a reduction in the number of hippocampal GR, as well as impaired negative feedback (Champagne, 2013), a desensitization of the GR and a shift from humoral immunity to that of cellular immunity, evident in a chronic inflammatory state (more on this later) (Elenkov & Chrousos, 2002; Luecken & Lemery, 2004; Miller, et al., 2009b).

Given the far reaching implications of childhood trauma/maltreatment on the backdrop of a continuum of anxiety states and traits, it is imperative to search not only for ways to optimise the environment, but also to find ways in which children "at risk" of development of anxiety can be a) identified, and b) taught to cope with adverse events or situations to improve their resilience to these debilitating maladjustments. Deeper understanding of specific associations between especially peripheral physiological parameters and neurological or psychological outcome/symptoms, may enable monitoring of treatment success to improve resilience by assessment of peripheral physiological parameters. Perhaps, in this situation, physiology, especially, has a niche in that it may be used to monitor progress in a psychologically less-invasive manner in traumatised populations. However, before such an attempt can be made, it is necessary to understand the processes involved and the nature of physiological adaptations to childhood maltreatment. On the more practical side, suitable reference ranges, or at least "ranges for change that would indicate either progression or regression" will have to be established.

**2.4.1 Developmental central sequelae: pathological asymmetry, limbic hyper-activation and lack of integration**

The neural foundation of anxiety is laid down as early as during infancy where, in the case of insecure attachment, environmental stimuli are appraised as threats to survival. In subsequent years, fear is further conditioned by pairing fearful thoughts, feelings or sensations with noxious stimuli. In addition, these conditioned responses are reinforced through the process of LTP, by which the association among neurons in these neural circuits become strengthened.

The amygdala is the primary locus of fear processing as well as appraisal of danger, safety, and familiarity (Berntson, et al., 2007; Sarter & Markowitsch, 1985). At the 8th month of gestation, the amygdala and brainstem are fully functional, allowing the foetus to experience intense physiological states of fear in response to, for example, distress experienced by the mother (Cozolino, 2010). In contrast, both the hippocampus (Kohman & Rhodes, 2013) and cortex are still immature at birth and only grow gradually throughout childhood to eventually organize and inhibit the amygdala (Alexander & Goldman, 1978; Cozolino, 2010; Fuster, 1980). During the first 2 years of life, information therefore reaches the amygdala independently of the cortex and hippocampus, a pathway which is equivalent to LeDoux's model of the fast system (Stevens, 2002).

In the relative absence of slow system functioning, infants are to a great extent dependent on caretakers for external modulation of the amygdala and subsequent emotional learning (Brodal, 1992). In addition, before the age of 3, there is a bias toward right hemisphere development, in favour to the left, with attachment, emotional regulation, and self-esteem organized predominantly in the right hemisphere (Chiron, et al., 1997; Schore, 2003). As a result, memories laid down during early childhood are implicit and reside in subcortical networks. They are also context free, emotional, and non-declarative, since language is processed in the cortex and left hemisphere (Tsoory, et al., 2008). The collection of these memories serve as a programmed paradigm reference framework for stimulus processing, accompanied by visceral, sensory-motor responses, as the amygdala has direct connections with the hypothalamus, limbic-motor centres and the brainstem, which upon activation results in somatic arousal (Stern, 1985; Stern, 1998).  This was clinically illustrated in 15 right-handed paediatric psychiatric inpatients with a history of abuse, where electroencephalogram (EEG) coherence indicated that their right hemispheres were significantly more developed than their left, with the left hemisphere development of the abused subjects lagging significantly behind that of healthy controls (Teicher, et al., 1997). In light of the above, it can be concluded that right hemisphere dominance occurs at the expense of left hemisphere functioning.

This lateralization involves relatively greater right prefrontal cortical activation in favour of the left when negative affective emotional stimuli are presented, resulting in less effective inhibition of the right amygdala (Amaral, et al., 1992; Gewirtz, et al., 1997; Morgan, et al., 1993). When this route is taken, information bypasses the hippocampus and reaches the amygdala, resulting in amygdala hyper-reactivity, which is frequently observed in patients presenting with PTSD, panic disorder and generalized anxiety disorder (Rauch, et al., 1996b; Shin, et al., 2005b; Taylor & Liberzon, 2007). This signifies conditioned fear acquisition and expression, with less potential for fear extinction, (Clark, et al., 2004; Davis, 1998; Morris, et al., 1999; Quirk,

et al., 2000; Rauch, et al., 2000; Romanski & LeDoux, 1992; Shin, et al., 2005b; Whalen, et al., 1998). Also, memories encoded within these networks during the time of trauma exposure are not open to deliberate recall but are instead accessed automatically *via* conditioned cues (Ehlers & Clark, 2000).

Thus, maladjustments in the developing brain also extend to connections between and activation of cortical and subcortical structures. As the medial areas of the prefrontal cortex expand and extend neurons to the limbic system and the brain stem during brain maturation, children would normally develop the capacity to regulate their emotions, impulses and attention (Diorio, et al., 1993; Teicher, et al., 2003). However, when this "top-down" processing is impaired it leads to deficits in affect regulation. Thus, as a result of childhood trauma and major life events, some brain structures may co-mature and connect more heavily with each other than other regions. Psychological and physiological wellbeing depends not only on the coherent integration of these structures, but also appropriate communication between the slow and fast systems for example, the amygdala is activated in favour of prefrontal regions, which ultimately results in dominance of the fast system during stimulus processing. This pattern of activation may persist into adulthood with continued negative life events serving to maintain insecure attachment (Hamilton, 2000; Kirkpatrick & Davis, 1994; Weinfield, et al., 2000) and determine an individual's affective style, which in turn increases vulnerability to develop psychopathology (Boscarino & Hoffman, 2007; Davidson, et al., 2000; Gray, 1990; Lang & Bradley, 2010; Lang, et al., 1990).

### 2.4.2 Neurocognitive maladaptations

As was evident from section 2.3.2, in the context of anxiety, stimulus processing takes place within the following frameworks, namely a) a disproportionate attention to threatening cues (selective bias); b) ambiguous aspects of current experience are resolved in a disproportionately threatening manner (interpretive bias), and c) situational cues are assessed based on threatening information from the past which is disproportionately accessible, i.e., there exists a memory bias which skews current experience in favor of danger assessment.

These frameworks can be assessed by means of symptom provocation, which is arguably the most widely used paradigm within neuroimaging studies focusing on anxiety. Structural imaging of various brain regions associated with information processing akin to the above selective, interpretive, and memory biases, can inform as to how these types of stimulus processing relate to long term morphological changes in the brain. In addition, specific neurocognitive deficits, also associated with pathological information processing characteristic of anxiety, may give a more comprehensive picture pertaining to the different cognitive processes (linked to specific brain areas) underlying information processing.

Neurocognitive tests are used to inquire about neurocognitive functions under emotionally neutral circumstances as opposed to those neuroimaging tasks that manipulate the emotional valence of the stimuli. Specific to the study of working memory, a meta-analysis has confirmed that verbal memory impairment (denoting left hemisphere deficits) is consistently present in adults with PTSD (Johnsen & Asbjørnsen, 2008). More specifically, neurocognitive tests have shown that in individuals with a history of psychological abuse, left-hemisphere neurological deficits were six times more prevalent than right-hemisphere deficits (for a review, see (Teicher, 2000)). In line with this, several authors have noted that patients with PTSD appear more likely to show deficits on verbal (left hemisphere function) than on visuospatial (right hemisphere) tasks (Brewin, et al., 2007; Danckwerts & Leathem, 2003; Toren, et al., 2000; Vasterling & Brailey, 2005). This was also the case in adults with histories of childhood physical and sexual abuse, with deficits displayed for verbal (and not visual) memory (Bremner, et al., 2004). However, these results pertain to cognitive functioning in the event of trauma exposure, with no distinction made between acquired deficits associated with years of maltreatment, *versus* neurocognitive deficits associated with pre-existing dispositions of anxiety, not modulated by trauma.

We have therefore also given an account of working memory (WM) in association with anxiety. Documented deficits on cognitive performance in relation to anxiety, independent of childhood trauma, is thought to be mediated by the impact of anxiety-biased processes associated with working memory, at both the level of storage and manipulation of information (Visu-Petra, et al., 2006). More specifically, a study has provided evidence for threat-induced anxiety (anxious arousal in anticipation of electrical shock) selectively disrupting accuracy of spatial but not verbal WM performance, and this was mediated by individual differences in physiological measures, as indexed by valence-sensitive corrugator EMG amplification and startle reflex potentiation. In the absence of threat (more representative of our study's neurocognitive assessment set-up), individuals characterized by high levels of behavioral inhibition (BI)- BI has been suggested to reflect a disposition to respond with state anxiety or anxious arousal in the event of performing a neurocognitive test- exhibited more intense anxiety and relatively worse spatial than verbal WM performance, indicating a mediating role of anxiety in disrupting specifically functions of working memory attributed to the right hemisphere (Shackman, et al., 2006). Both these accounts support the general notion of anxious arousal selectively disrupting spatial working memory, which is likely to be mediated by competition between task-relevant and anxiety relevant goals for limited visuospatial attention resources localized to the right PFC (Lavric, et al., 2003). Therefore, when interpreting deficits in neurocognitive function as a consequence of task-irrelevant anxiety depleting central executive resources otherwise available to support WM, it is important to distinguish

deleterious effects of anxiety primarily mediated by anxious arousal, *versus* anxiety due to the trait characteristic of anxious apprehension. To note, our high anxiety prone groups displayed generally high levels of anxious apprehension (trait anxiety), and high levels of anxious arousal only when provoked.

Turning to the rationale for including measures of more global executive function in the current study, it has been suggested that anxiety is associated with a depletion of central executive resources (e.g., (Eysenck & Calvo, 1992). In context of executive function as well as working memory specifically, Baddeley's model (Baddeley, 2000) states that inhibition to inaction of behaviour is thought to be due to working memory providing a delay during which executive processes can set in (Wilson, et al., 2011). Inhibition, more specifically, allows for attention to the stimulus where attention must be maintained long enough for ample information about the stimulus to be collected into the working memory. Therefore, working memory is the process that stores information temporarily, keeping it handy for quick reference (Dennis, 2006) and executive functions enable one to control one's behavior *via* the following processes: a) volitional activity, which relies on self-awareness, initiation, and motivation; b) planning and organization; c) carrying out purposive action; and d) self-regulation, which relies on monitoring, shifting, inhibiting, and self-correcting (Lezak, 1995). Accordingly, proper integration of memory content is reliant on executive functions (Mesulan, 2000; Royall, et al., 2002).

On a neurological level, executive functions have been primarily located to the frontal lobes, specifically the PFC, where the PFC is considered the shut-off valve for the stress response in the HPA-axis, given that the PFC is functioning properly (Wilson, et al., 2011). Furthermore, executive function is also associated with processes reducing conditioned fear, *via* engagement of the medial prefrontal cortex (Milad & Quirk, 2002; Shin, et al., 2005a) and executive deficits commonly observed in PTSD has been associated with the dorsolateral prefrontal structures (Leskin & White, 2007). We therefore propose that poor executive function would be associated with more amygdala based fear processing, which in turn would lead to activation of the HPA-axis.

With regard to research on anxiety and neuroendocrine function, studies employing neurocognitive tests on executive function have largely focused on trauma exposure and not much is known in relation to neurocognitive deficits associated with sub-clinical anxiety (in the absence of PTSD diagnosis). Childhood maltreatment has been shown to lead to overall deficits in neurocognitive functioning in children (De Bellis, et al., 2013), and specifically, deficits in executive functions (Kavanaugh & Holler, 2014; Mezzacappa, et al., 2001; Perna & Kiefner, 2013), and working memory (e,g. (Beckham, et al., 1998)) in children, as well as verbal declarative memory in adults (delayed recall; (Bremner, et al., 2004). The type of maltreatment also

needs to be considered, as a prospective study found that childhood maltreatment, overall, and childhood neglect, specifically, predicted poorer executive functioning in middle adulthood, whereas physical and sexual abuse did not (Nikulina & Widom, 2013). Also, a history of trauma-exposure (particularly emotional neglect), rather than the number of abuses experienced, impacts on neurocognitive functioning in traumatized children and adolescents (Revington, et al., 2011). In addition, in children, community stressors were not correlated with neurocognitive deficits when viewed relative to maltreatment (Fishbein, et al., 2009).

**2.4.3 Brain volumetric maladaptations**

As reviewed in section 2.4.1, individuals with a history of childhood maltreatment exhibit more developed right hemispheres in comparison to their left and relatively greater right prefrontal cortical activation in favour of the left when negative affective emotional stimuli are presented, which is associated with less effective inhibition of the right amygdala and predominantly amygdala hyper-reactivity. However, in the context of anxiety, discrepancies exists in the literature pertaining to brain activity in the left hemisphere, compared to the right, with articles reporting either more right or more left hemisphere activity. A physiological basis may underlie these discrepancies. Accordingly, evidence points toward a distinction between anxious apprehension (worry, cognitive anxiety, anticipatory anxiety, anticipatory frustration) and anxious arousal (somatic anxiety with physiological hyperactivity), where, patterns of hemispheric asymmetry in electroencephalogram alpha showed more right than left activity in the anxious arousal group and no significant asymmetry in the anxious apprehension group (Nitschke, et al., 1999). It is also important to note that this finding was evident in measures taken in the absence of an affect provocation, i.e. in the absence of challenge in the form of presenting fear associated cues and/or emotionally-valenced imagery. It might well be that increased right hemisphere function seen in studies using these types of challenges might be due to the direct effects of anxious arousal and not due to trait anxiety, *per se*. This is also in line with the afore mentioned study and commentary in section 2.4.2, where anxious arousal selectively disrupted spatial working memory associated with the right PFC. Therefore, in functional magnetic resonance imaging (MRI) studies investigating the effects of anxiety due to anxious apprehension, it may not be as useful to examine left *versus* right hemisphere function, due to the possible confounding factor of anxious arousal in response to the experience of being in the MRI scanner. We suggest rather investigating LeDoux's short system *versus* long system in this instance.

When the long system is inhibited during the processing of negative stimuli, the short system predominates in the form of hyper-reactivity in brain regions associated with emotion recognition and generation of emotional

reactions (such as the amygdala), with concomitant hypo-activity in regulatory circuits (such as the prefrontal cortex) (Hofmann, et al., 2012). This scenario not only pertains to the biased selective attention to threat and interpretation of emotionally ambiguous stimuli, but also serves to perpetuate anxiety in the acquisition of conditioned fear and failure of the extinction thereof.  Accordingly, in a functional MRI study investigating trait vulnerability to anxiety, the authors found evidence for a lesser capacity for the extinction of conditioned fear in high trait anxious individuals, supported by increased amygdala responsivity to phasic fear cues as well as impoverished ventral prefrontal cortical (vPFC) recruitment to down-regulate both cued and contextual fear prior to extinction of the aversive unconditioned stimulus (Indovina, et al., 2011).

However, in terms of structural MRI studies in relation to anxiety and trauma, it is important to look into left *versus* right volumetric differences in the hemispheres of high and low trait anxious individuals, as that could give an indication of morphological differences in the over- or under-development of specific brain regions, in response to anxiety and/or childhood trauma. Taking this approach may also indicate whether larger volumes of left or right hemisphere  is implicated in resilience, when comparing  a group with high trauma exposure and anxiety with one of high trauma exposure and low anxiety, in relation to physiological parameters.

This is based on the notion that the immune system is linked to the central nervous system *via* the HPA axis, and it has been postulated that the right prefrontal cortex controls the activity of the left, which in turn modulates the immune system (Renoux & Biziere, 1986; Renoux, et al., 1983). Indeed, it has been suggested that asymmetrical brain activation results in differential immune responsiveness, as well as immune-homeostasis (Kim, et al., 1999; Lawrence & Kim, 2000).

More specifically, right brain dominance may result in dampened peripheral immune responses: lesions to the left but not the right cortex caused inhibited NK activity (Bardos, et al., 1981) and IL-2 (but not IL-1) production (Neveu, 1992). T cell function has also been found to be controlled asymmetrically (Renoux, et al., 1983). In addition, electroencephalography (EEG) analysis has revealed significantly lower NK cell activity and higher serum Immunoglobulin E (IgE) levels in woman with extreme right frontal activation in favour to the left (Kang, et al., 1991). Furthermore, a significantly bigger proportion of dextral compared to non-dextral individuals were reported to have resting serum IL-6 levels above the lower limit of the assay sensitivity (Chengappa, et al., 1994). A two-way communication loop between the brain and the immune system has also been proposed, with the immune system feeding back to the brain in a lateralized fashion (Abramov, et al., 2006; Neveu, 1992). Although these studies were not presented in the context of anxiety or trauma, it poses an

interesting possibility that brain lateralization may influence immune resilience in the face of anxiety and/or trauma.

Although the literature on structural differences between the left and right hemisphere is limited, in context of anxiety in the absence of clinical diagnosis, left amygdala volumes predicted anxiety, with decreased left amygdala volume associated with higher anxiety in healthy adults, as indexed by both state and trait anxiety measures (Blackmon, et al., 2011), and right hippocampal volume was positively associated with greater levels of behavioural inhibition (BIS) (Levita, et al., 2014). Furthermore, specifically prefrontal right brain dominance, has been associated with higher BI (Shackman, et al., 2009; Sutton & Davidson, 1997) and anxiety (Davidson, et al., 1990; Levita, et al., 2014), albeit by means of assessing neural asymmetry in scalp EEG, as opposed to a structural MRI.

Turning to the effects of childhood maltreatment on brain asymmetry: to our knowledge, no study has investigated trauma in the absence of PTSD. For lack of better references, we refer to a study that has reported a reduction in left hippocampal volume in adults with childhood trauma and a current diagnosis of PTSD or dissociative identity disorder (Pedersen & Vedeckis, 2003). In studies using EEG coherence, right-handed psychiatric inpatients with a documented history of abuse, exhibited right hemispheres that were significantly more developed than their left, and the right hemispheres of abused subjects had developed to the same degree as the right hemisphere of controls, while the left hemisphere of the abused subjects lagged substantially behind the left hemisphere of healthy controls (Ito, et al., 1998; Teicher, et al., 1997).

**2.4.4 Potential outcome modulating factors of neurophysiological maladaptations**

Before moving on to reviewing the literature regarding maladaptive neuro-endocrine sequelae, it is important to consider factors modulating these maladaptive responses to anxiety and/or childhood maltreatment. In context of the current thesis, we have identified three potential outcome modulating factor (OMFs) in the aetiology of neurophysiological (mal) adaptations. These are resilience, handedness, and self-esteem, for which background information is provided in the next three sections.

**2.4.4.1 Resilience**

In the current study, in addition to identifying specific markers of resilience associated with absence of psychopathology despite exposure to trauma, resilience was also included as a quantified parameter, by means

of a questionnaire. This measure served as a complimentary indication of resilience so as to identify specific role players of resilience in terms of immune and endocrine parameters, by means of correlational analysis. Despite the well documented deleterious consequences of childhood maltreatment on mental health, there is evidence that many children who have been maltreated succeed in overcoming some of these possible consequences and are deemed resilient (for a review, see (Klika & Herrenkohl, 2013)). Resilience is characterized as the ability to bounce back from negative experiences through flexible adaptation to changing life demands (Lazarus, 1993; Luthar, et al., 2000; Masten, 2001). Resilience can also be defined as the absence of psychopathology despite exposure to extreme life stressors (Bonanno, et al., 2007; Tiet, et al., 1998). Agaibi and Wilson (Agaibi & Wilson, 2005) defined resilience as a "complex repertoire of behavioral tendencies or adaptation to situational stress" and "a style of personality functioning, including resistance to and recovery from psychopathology". Thus, vulnerability identifies risk factors eventuating in pathology, whereas resiliency identifies physiological markers leading to positive adaptive behavior (Garmezy, 1991).

It has been agreed that, specifically pertaining to abuse-related resilience, a working definition of resilience should include a) sufficient levels of "risk" so as to "facilitate" resilience, and b) markers of resilience should be multimodal and be evident across an extended period of time (Collishaw, et al., 2007). Accordingly, the conclusion drawn from a systematic review of longitudinal studies on childhood sexual abuse, is that resilience is a product of a combination of factors, where markers of resilience should not be viewed in isolation, but rather in conjunction with possible confounding variables such as social support and community factors (Herrenkohl, 2013; Marriott, et al., 2014).

Of clinical relevance is the notion that once biomarkers of resilience have been identified, it can be used to predict (if trait-related) or track (if state-related) recovery from traumatic events.  Trait refers to the ability to bounce back, whereas state can be viewed as the process of adapting, or the product of successful adaptations. Trait-related capacities for adaptation may be pre-traumatic predictors of resilience whereas state traits may be acquired as a result of an arising necessity to cope with adversity (Yehuda & Flory, 2007). We therefore suggest that by identifying markers of resilience, more effective intervention strategies can be developed, targeting the promotion of resilience on both psychological and physiological platforms.

Considering self-esteem -a variable predictive of resilience and which is also considered an inner resource-, it has been utilized as an outcome measure in research on childhood maltreatment (Feiring, et al., 1998; Runtz & Schallow, 1997). However, whether self-esteem should be regarded a precursor of resilience or an outcome, or

both, is still not clear (Jonzon & F., 2006). Another issue to bear in mind when assessing inner resources in association with resilience is the argument that much of the research is based on Western literature and concepts (Ungar & Liebenberg, 2011). Another marker of resilience is handedness, where consensus yet remains to be reached pertaining to whether being left-, right-, or mixed-handed contributes to resilience in terms of psychopathology (e.g. (Prichard, et al., 2012)).

### 2.4.4.2 Handedness

As reviewed in section 2.4.1, in the context of trauma exposure and/or anxiety, right hemisphere processing of anxiety provoking stimuli takes precedence to that of the left. However, other than the fact that brain asymmetry appears to be weaker and more variable in left-handers, research attempting to identify key functional and structural differences between left- and right-handers has produced ambiguous results (Prichard, et al., 2012). More specifically, excluding the transient laterality of brain function under conditions of acute stress/challenge (as investigated by functional MRI studies), not much is known in context of the overall disposition of brain asymmetry associated with lateralization of cognitive function, under emotionally neutral conditions (especially in the case of high anxiety prone individuals).

Hand dominance has been considered an-easy-to-measure proxy of brain asymmetry, given the association between handedness and cerebral dominance, which has been, in turn, closely correlated with anatomical asymmetry (Sommer, et al., 2001). In 100 volunteers, 96% of right-handers and 76% of left-handers displayed left-hemisphere language dominance (Pujol, et al., 1999). Another measure associated with handedness is that of behavioral laterality which has been found to predict emotional lateralization (Elias & Bryden, 1998). In the past, research on handedness has focused on the direction of hand preference, i.e., the direction of left *versus* right handedness. However, recent advances in research based on laterality suggest that instead of comparing left- versus right-handedness, it is required to focus on comparisons between consistent/strong-handers (CH) and inconsistent/mixed-handers (ICH), where CH use the dominant hand for virtually all common manual activities, and ICH use the non-dominant hand for at least one common manual activity (Prichard, et al., 2012). Thus, a more accurate representation of handedness is based on the degree of hand preference (consistent *versus* inconsistent), which is in accordance with measures of the Edinburgh Handedness Inventory (EHI) used in the current study.

In the context of handedness in association with anxiety, research is sparse. We found a single study of healthy subjects in which left-handedness was associated with greater anxiety than right-handedness (but only among

inconsistent left-handed individuals) and consistency (being consistently left or right handed) was associated with greater anxiety than inconsistency (but only among right-handers) (Lyle, et al., 2013). Perhaps because there is more speculation than original data in the available literature, there has been little agreement between researchers as to how far the empirical data support any link between handedness and cognitive skills in normal populations,  (Bishop, 1990; Mellet, et al., 2014). The majority of studies have provided conclusions on cognitive performance, based on either left- or right- handedness, and have not paid much attention to the range in between.  Nevertheless, one study has found that compared to strongly handed individuals, mixed-handedness is associated with a lower threshold for updating beliefs in response to information inconsistent with those beliefs, due to increased inter-hemispheric interaction between the left hemisphere – the left hemisphere maintains current beliefs, and the right hemisphere evaluates and updates those beliefs when appropriate (Niebauer, et al., 2004). This finding suggests interplay between the two hemispheres in functions of appraisal, further emphasizing the potential importance of assessing degree of handedness in neurocognitive studies. Further gaps in the literature include links with anxiety and childhood maltreatment, with the aim of establishing whether handedness could be used as a marker of resilience/risk in the event of trauma exposure. However, before such conclusions can be made on handedness in relation to psychopathology, it is important to establish how accurately handedness can be used as a measure indicating cognitive lateralization.

Consensus still has not been reached as to whether handedness, along with cerebral lateralization - related to language and behavioral/emotional lateralization- and differences in cognitive performance are predominantly functions of genetics, or instead the environment. Both genetic and non-genetic explanations of individual differences in handedness have been put forward. Even with the acceptance of a hereditary component in the 90% bias toward right-handedness in the general population, this bias has been proposed to be probabilistic rather than deterministic, so that environmental factors, as well as cultural transmission of hand preference between generations, can overrule it (Laland, et al., 1995). For example, exposure to adverse environments and pathogenic insults, such as low birth weight, birth stress and early-life excessive exposure to ultrasound, has been postulated to increase the prevalence of left-handedness in the healthy populations (Bailey & McKeever, 2004; Bakan, et al., 1973; Salvesen, 2002; Satz, et al., 1985). For many years, twin studies have been performed in an attempt to clarify the relative contribution of genes *versus* the environment in hand dominance. These studies either revealed discordant handedness in identical twins (Bishop, 2001), weakening the argument for genetic control, or, as a meta-analysis of 35 studies has pointed out, have yielded disparate results due to small sample sizes and methodological problems regarding how to define handedness (Medland, et al., 2006).

Nevertheless, the same meta-analysis argued for the AE model (no effect of shared environment) to provide the best fit of the data of twin studies, and yielded estimates of only 25.9% for the proportion of variance in handedness in twins to be due to additive genetic effects and 74.2% due to unique environmental effects. The same research group has reproduced this finding in a large sample of twins and their siblings (54,270 individuals), with 24% of sample variance attributed to additive genetic effects, while the remaining 76% was due to non-shared environmental influences (Medland, et al., 2006).

Although the above findings do not support the view of handedness being a hereditary trait, it does not necessarily exclude the possibility of genetics in the etiology of handedness. For example, rather than distinguishing between right-handedness and left-handedness when studying the relative contribution of nature *versus* nurture in handedness, right-handedness and lack of bias to either side can be the focus (Annett, 1985) (McManus, 1985). The right-shift theory has been proposed by Annett, and states that handedness depends on a single autosomal gene with the two alleles, rs- and rs+ (Annett, 1985). A rs++ genotype results in a strong bias to right-handedness (along with left cortical lateralization) while a rs+- genotype denotes a  more moderate bias to right-handedness, and individuals displaying the genotype rs-- have no bias to either side. In this scenario, random environmental factors influence handedness in all individuals; however, in those with a single or double rs+ allele, synergy between these genes and random environmental factors causes a bias toward more right-handedness, accordingly allowing for two identical twins to present with discordant hand preference. Annette's theory also proposes that mixed handed individuals present with an evolutionary advantage to their pure left and right handed counterparts. However, this theory has not been the focus of subsequent research and remains largely untested and unsubstantiated.

**2.4.4.3 Self-esteem**

Self-esteem is the evaluative aspect of self-knowledge that concerns the extent to which people like themselves (Brown & Marshall, 2006), and is a relatively enduring characteristic that possesses both motivational and cognitive components (Kernis, 2003). Moment-to-moment perceptions of rejection and acceptance has been linked with moment-to-moment changes in *state* self-esteem *via* associations with social rejection-related neural regions (dorsal anterior cingulate (ACC) and anterior insula) and mentalizing regions (Eisenberger, et al., 2011). Although this finding provides a neurological locus for self-esteem, this focus is limited to a transient function of self-esteem. We provide an account of *trait* self-esteem, with self-esteem measured by the R-SES signifying a

single personality dimension, although its function as a one-dimensional scale has been questioned (Hensley & Roberts, 1976).

Trait self-esteem has showed substantial continuity over time, albeit with some variation: low during childhood, increasing throughout adolescence and young adulthood, and declining during midlife and old age (Robins, et al., 2002; Trzesniewski, 2004). Low self-esteem has been linked with a need for control (Burger, 1995) and with anxiety (Henning, et al., 2007), with the latter associated with specifically contingent self-esteem (Crocker & Wolfe, 2001), which is based on the belief that one must do certain things or be a particular type of person in order to have worth as an individual (Bos, et al., 2010). Furthermore, attachment anxiety has been associated with self-esteem instability (Foster, et al., 2007) which refers to fluctuations in the moment-to-moment feelings of self-worth reported by individuals (Kernis, 2003). More specific to the current study, in adolescents, studies have found an inverse relationship between self-esteem and levels of state/trait anxiety (Fickova, 1999; Newbegin & Owens, 1996).

Additionally, in young adults, significant indirect effects of trait anxiety and trait resilience on self-esteem have been reported, with a mediating role of positive and negative affect, respectively (Benetti & Kambouropoulos, 2006). Conversely, the reverse has also been posited, where low self-esteem is a strong predictor of personality disorders and psychopathological symptoms (Erol, et al., 2002). In the same vein, self-esteem can provide buffering from anxiety as it is the prerequisite for feeling loved, safe, secure and supported by life itself. Indeed, three experiments by Greenberg and colleagues (Greenberg, et al., 1992) have provided evidence for the anxiety-buffering effects of self-esteem. Answering the question of whether low self-esteem is a cause or consequence of anxiety, would suggest whether self-esteem or rather anxiety should be the primary and/or initial focus in therapy.

Mechanisms describing the interplay of self-esteem and anxiety may include theories of attachment based on associations between secure and dismissive attachment styles and higher global self-esteem as measured by the Rosenberg scale (Blysma, et al., 1997; Huntsinger & Luecken, 2004; Schmitt & Allik, 2005). However, caution should be taken when  attempting to pinpoint which items on the scale are correlated with which specific attachment styles (Mannarini, 2010), and further investigations of linking attachment to measures on the R-SES are required. More impetus for exploring this link involves the notion that attachment formed during infancy, can play a significant role in an individual's conceptualization of self and others in future relationships during childhood and adolescence. Indeed, a 12-year longitudinal study has provided evidence for infant attachment

style predicting attachment style in adolescence (Hamilton, 2000) and a meta-analysis displaying moderate stability of attachment across the lifespan (weighted r = 0.27 from infancy to 19 years of age; (Fraley, 2002). Accordingly, in adolescents, an association between insecure attachment and low self-esteem, dysfunctional attitudes, and negative attributional style was evident (Gamble & Roberts, 2005). Similarly, an association between insecure attachment and anxiety symptoms has been found again in adolescents (Muris & Meesters, 2002) as well as adults (Hankin, 2005; Safford, et al., 2004).

In broad terms, insecure attachment is comprised of anxious attachment, characterized by anxiety and fear of rejection, and avoidant attachment, characterized by discomfort with closeness. Anxious attachment has been specifically associated with anxiety (Eng, et al., 2001). In addition, self-esteem was considered to be a mediator in the association between baseline anxious attachment and prospective increases in anxiety symptoms, in effect providing support for the hypothesis linking anxious attachment to dysfunctional attitudes about self and others, and, in turn dysfunctional attitudes to lowered self-esteem, and finally, lowered self-esteem to later internalizing symptoms such as anxiety (Lee & Hankin, 2009).

Stepping away from self-esteem in relation to anxiety alone, and equally relevant to our study, is the issue surrounding how childhood maltreatment shapes self-esteem, and how self-esteem, in turn, may buffer against the effects of maltreatment. The vulnerability model states that self-esteem and stress interacts to influence the occurrence of psychopathology: high self-esteem buffers against the deleterious consequences of stress, whereas low self-esteem increases vulnerability to the effects of stress (Zeigler-Hill, 2010), and this model tends to be best supported when the outcomes measured are physical symptoms and anxiety (Baumeister, et al., 2003). In contrast, the scar model posits that low self-esteem is a consequence of psychopathology due to stress, rather than one of its causes, although the vulnerability model and the scar model are not mutually exclusive (Harter, 1999).

Theories on mechanisms by which childhood maltreatment can influence evaluations of the self, and subsequently self-esteem, include two aspects of the self, namely, the self as subject (the I-self) and the self as object (the Me-self) (Harter, 1983; James, 1890; Lewis & Brooks-Gunn, 1979). The I-self is the self that realizes that it exits separate from others. More specifically, the I-self is a) the actor, knower, or observer, b) an active agent organizing and interpreting one's experience, and exhibits, c) self-awareness, d) self-agency (the sense of the authorship over one's thoughts and actions), e) self-continuity (the sense that one remains the same person over time) and f) self-coherence (the stable sense of the self as a single entity). On the other hand, the Me-self is

categorical in that it creates categories by which to define the self and refers to a) the object of one's knowledge (the observed), b) represents the empirical aggregate of what is objectively known about the self, and c) involves a 'theory' that is constructed to organize one's thinking about one's relationship to the social world. It is the I-self that gives rise to self-esteem *via* Me-self functions, and childhood maltreatment can lead to disturbances in both I-self and Me-self processes, with especially disturbances in the Me-self. This puts maltreated children at multiple risks for behavioral and psychological maladjustment (for a review, see (Cicchetti & Rogosch, 1994), particularly following the argument that self-esteem is created through the incorporation of the attitudes and evaluations that others, particularly parents in early childhood, hold toward the self (Harter, 1999).

In 6-11 year olds, a multivariate growth modeling indicated that, physical abuse was negatively related to levels of self-esteem (Kim & Cicchetti, 2006) and emotional abuse in childhood (Mullen, et al., 1996). The effects of childhood maltreatment on self-esteem also extend into adulthood, where, evidence was provided for the long-term consequences of child abuse on self-esteem (Gross & Keller, 2006). Especially controlling and domineering methods of parenthood resulting in psychopathology was shown to be mediated by low self-esteem in adulthood, as a function of immature defense organization and damaged self-representation (Finzi-Dottan & Karu, 2006). In a longitudinal study on children, the effect of childhood maltreatment on self-esteem was mediated by the timing, type, and severity of maltreatment, with more chronic and severe maltreatment, specifically sexual abuse (in contrast to emotional abuse) predicting lower levels of self-esteem (Bolger, et al., 1998). It appears that the critical period for deleterious insults on self-esteem due to maltreatment is before the age of 11 (Moran & Eckenrode, 1992).

Taken the above sections in Part A together in terms measured variables in the current thesis, and how by doing this, it may contribute to the precision medicine paradigm, we a) identified amygdala hyperactivity as well as poor executive function to be markers of risk in the context of anxiety and/or trauma, b) highlighted gaps in the literature pertaining to the three investigated potential modulating factors (resilience, handedness and self-esteem) in the context of anxiety and childhood maltreatment, c) proposed self-esteem and resilience as avenues to address by means of intervention, and d) proposed investigation of the potential modulating factors in the establishment of risk/vulnerability markers. On the latter point, some of these factors – such as handedness – are difficult to modify therapeutically, however, these may have significant diagnostic value. In addition, it is also necessary to consider other systems that may be relatively more readily manipulated, in order to better long-term prognosis in this population. However, as is clear from Part A, the gaps also extend to the lack of

research on these factors in relation to physiological parameters. The relevant literature on neuro-endocrine and immune system adaptation will be addressed in this context in the second part of this chapter.

**Part B: Maladaptive neuro-endo-immune sequelae to trauma and anxiety**

Given the very limited number of relevant published studies on adolescent population, the next section will take a somewhat broader approach and include studies on adult populations, as well as clinically pathological extremes, to illustrate maladaptive processes.

**2.5 HPA-axis function**

Cortisol suppression in response to dexamethasone (Dex) or interchangeably, betamethasone, denoting synthetic glucocorticoids with high affinity to the GR, gives an indication of the level of responsiveness of the GR, as well as the degree of negative feedback inhibition at the level of the pituitary. Measuring levels of ACTH as well as cortisol post Dex and CRH administration enables to distinguish whether hypocortisolism is due to insufficient hormone release at the level of the pituitary and/or adrenal cortex, respectively. Both the Dex suppression test and the Dex/CRH test have proved to be useful in assessing HPA axis function in subjects who have not yet manifested with clinical symptoms of PTSD, and may serve as a tool to detect endophenotypes at risk for developing PTSD as well as other neuropsychiatric disorders (Hasler, et al., 2004). Another test used to assess HPA axis responsivity is the Trier Social Stress Test (TSST), a standardized psychosocial stress challenge.

It has been suggested that inter-individual variation in neuroendocrine stress regulation represents risk or resilience in the development of stress-related disorders (Binder & Holsboer, 2012). For example, it has been shown that sufficient cortisol in the immediate aftermath of an acute trauma protects against the development of PTSD (Briegel, et al., 1999; Delahanty, et al., 2000; Sapolsky, et al., 2000). However, unlike the extensive research on HPA-axis perturbations in depressive disorder, less is known about anxiety disorders and HPA-axis activity on the backdrop of childhood maltreatment, and the existing literature is inconclusive (Vreeburg, et al., 2010).

**2.5.1 HPA-axis maladaptation in PTSD**

When investigating endocrine and immune profiles as a function of anxiety and/or trauma, we have first considered general neuro-endocrine and –immune trends observed in the context of PTSD, since much research

has been done on this condition. By first investigating HPA-axis function in a clinical setup, data from studies involving trauma and anxiety in the absence of PTSD diagnosis could then be interpreted in light of a) trauma- and anxiety-induced physiological profiles lying somewhere on a continuum of physiological perturbations, with PTSD at the extreme end, or b) anxiety/trauma physiological profiles indicating distinct phenotypes unlike what is observed in the context of PTSD.

The literature provides discrepant findings specific to the study of PTSD and HPA-axis activity, which may reflect, for example, individual differences in gender, trauma type, stage of development at trauma occurrence (e.g., childhood vs. adulthood), and early pre-traumatic risk factors (Klaassens, et al., 2012; Metzger, et al., 2008). In order to maintain a degree of homogeneity pertaining to pooling data of patients with PTSD with characteristics similar to our study population, we included only those studies on PTSD in adults who have been exposed to long standing traumatic events (as opposed to one or two major life events), where the nature of the trauma has been either that of combat (veterans), living in exile (refugees), sexual or physical abuse, or domestic violence. Accordingly, the following consistent reports have been observed:

a)  With the exception of a few studies (Inslicht, et al., 2006; Lemieux & Coe, 1995; Lindley, et al., 2004) low basal cortisol levels have  been consistently reported (Bauer, et al., 2010; Lindleya, et al., 2004; Mason, et al., 1986; Neylan, et al., 2003; Rohleder, et al., 2004; Thaller, et al., 1999; Wessa, et al., 2006), although a systematic review and meta-analysis revealed that low cortisol levels do not relate to PTSD in general, but rather seem to mirror PTSD subgroups based on type of trauma and gender, where lower basal cortisol levels have been reliably associated with PTSD due to physical or sexual abuse (Meewisse, et al., 2007).

b) Low awakening cortisol responses (Lauc, et al., 2004; Wessa, et al., 2006), especially associated with chronic PTSD, in contrast to PTSD severity (Johnsona, et al., 2012), indicating a time dependency rather that severity of insult for development of this hyporesponse (which interestingly, is also the case for cortisol hyporesponsiveness in the context of overtraining in athletes (Robson, et al., 1999).

c)  Enhanced mononuclear leukocyte glucocorticoid responsiveness/sensitivity centrally – evident in the enhanced *in vivo* suppression of GR number after administration of low dose Dex- (Yehuda, et al., 1995), as well as peripherally – evident from *in vitro* Dex assays- (Rohleder, et al., 2004; Yehuda, 2001; Yehuda, et al., 2004), and higher levels of GR expression in peripheral mononuclear leukocytes (Stein, 1997; Yehuda, et al., 1991) where it has been postulated that a high GR number in peripheral blood mononuclear cells (PBMCs) may be associated with increased risk for developing PTSD (van Zuiden, et al., 2013).

d) High cerebrospinal fluid (CSF) CRH levels (Baker, et al., 1999; De Kloet, et al., 2007; Geracioti, et al., 2008; Kasckowa, et al., 2001).

e) Heightened cortisol (Griffin, et al., 2005; Lindleya, et al., 2004; Stein, et al., 1997; Yehuda, et al., 1995) and ACTH (Newport, et al., 2004; Yehuda, et al., 2006b) suppression following 0.5 mg or 0.25 mg of Dex administration, suggesting a relative non-responsiveness of the lower (pituitary-adrenal) HPA-axis and/or up-regulated negative central feedback at the level of the pituitary.

f) Low grade inflammation due to polarization towards a T helper cell 1 (Th1) immune profile (Altemus, et al., 2003; Boscarino & Chang, 1999).

Considering the above scenario of increased CSF CRH levels co-occurring with low cortisol levels and enhanced Dex suppression, it appears that at least in PTSD, hypoactivity and/or hypoactivation of the lower (pituitary to adrenal gland) HPA-axis exists. Seeing that research focussing on anxiety and/or trauma outside the context of PTSD does not generally included a measure of CRH and therefore does not allow conclusions to be drawn for all levels of the HPA-axis, for the remainder of the thesis we will refer to relatively lower cortisol levels as being representative of lower HPA hypoactivity, so as to be more conservative in our investigation of the distinct phenotype(s) underlying anxiety and/or trauma exposure.

In terms of PTSD in children, only a few studies are available to review. In these studies, children diagnosed with PTSD and with a history of childhood maltreatment consistently exhibited cortisol levels which are high or normal in the majority of cases (Carrion, et al., 2002; Cicchetti, et al., 2003; De Bellis, et al., 1999a; De Bellis & Thomas, 2003). This is in contrast to what is seen in adult PTSD (see previous paragraph), but together suggest – similar to the notion of time-dependency of cortisol reactivity in (b) above – that the development of blunted HPA-axis responsiveness or capacity, is an effect that develops over time, and downstream of psychological changes used in diagnosis of PTSD. This supports the theory that nurture affects nature in a one-directional fashion.

Other mechanisms proposed for the above findings of a combination of high basal levels of CRH in CSF and low plasma cortisol levels in patients with PTSD (which contrasts with results seen in major depressive disorder patients) as well as enhanced Dex suppression and relatively high GR expression, include a) enhanced GC feedback sensitivity at the levels of the pituitary, b) pituitary insufficiency or reduced pituitary sensitivity to CRH, and c) adrenal insufficiency or reduced adrenal sensitivity to ACTH (De Kloet, et al., 2006) However, the

particular selection and combination of these mechanisms coming into play at a particular point in time during the lifespan of an individual suffering from PTSD, remain to be elucidated. This task is complicated by the fact that in most studies on PTSD, no indication is provided on the amount of time which has passed since the onset of trauma exposure. Accordingly, PTSD has been proposed to not be reflective of an acute response to stress, or of a response associated with a chronic stressor, but rather a persistent response to a challenge that is no longer present (Daskalakis, et al., 2013).

Therefore, it is still not clear whether dysregulation of the endocrine and immune systems in PTSD reflect an end-state scenario pertaining to anxiety, or whether PTSD presents with unique profiles, independent to those associated with individuals who have been exposed to chronic trauma, but did not go on to develop PTSD. Adding to the complexity of PTSD, a recent article has proposed two clinically and biologically distinct HPA-axis reactivity subgroups of PTSD:  One subgroup ("non-responders") showed a blunted HPA-axis response and distinct clinical and biological characteristics such as a higher prevalence of trauma-related dissociative symptoms as well as alterations in the expression kinetics of the genes encoding for the mineralocorticoid receptor (MR) and for FK506 binding protein 51 (FKBP51), when compare to a PTSD "responsive" subgroup (Zaba, et al., 2015). It is therefore not feasible to draw more general conclusions from the PTSD literature alone.

### 2.5.2 HPA-axis maladaptation with trauma exposure in the absence of PTSD diagnosis

Moving on to available data on childhood trauma and anxiety in the absence of clinically diagnosed anxiety, the literature is limited and available reports are contradictory. Since the scope of the current thesis involved repeated trauma/maltreatment experienced in childhood, with maltreated children not developing PTSD or other anxiety disorders during adolescence and adulthood, but instead developing more "lower grade" anxiety disorders or no disorder at all, it is imperative to investigate different endophenotypes of cortisol dysregulation associated with childhood maltreatment only, i.e. in the absence of other pathology or psychiatric diagnoses. In terms of the focus of this thesis, we have identified large gaps in the literature. The mechanisms of differential neural adaptation leading to these different outcomes remain to be elucidated, as current studies are contradictory and as a whole, inconclusive. Part of the reason for this is the use of different study designs: for example, while most studies on adults exposed to childhood maltreatment have focused on HPA-stress reactivity to a psychosocial stress test or to the effects of Dex/CRH administration (Carpenter, et al., 2007; Elzinga, et al., 2008; Newport, et al., 2004; Stein, et al., 1997; Watson, et al., 2007) studies in children mainly report basal cortisol levels  (Cicchetti, et al., 2010; Cicchetti & Rogosch, 2001; Greaves-Lord, et al., 2007; Saltzman, et al.,

2005). Furthermore, other confounding factors, such as anxiety proneness and resilience, are usually not included as co-variants in the interpretation of results. This makes the drawing of an integrated conclusion from results presented by these types of studies nearly impossible, especially considering the fact that most studies investigating childhood trauma do not include assessment of anxiety and/or anxiety proneness levels, with the latter possibly exhibiting a mediating effect.

One of the few studies investigating childhood trauma in combination with anxiety, measured longitudinal outcomes of childhood maltreatment before adoption, as well as the presence of internalizing (anxiety and/or mood) disorders and cortisol secretion in adulthood, in a cohort of 429 international adoptees: severe maltreatment before adoption modified the relationship between internalizing disorders and cortisol secretion in adulthood. For example, in adoptees diagnosed with an anxiety disorder, an added history of severe maltreatment was associated with lower daily cortisol secretion, while in adoptees without an anxiety disorder, no difference in cortisol secretion was observed between persons with and without a history of severe maltreatment early in life (van der Vegt, et al., 2010). This may suggest that pre-existing anxiety proneness may contribute relatively more to long-term HPA-adaptation than trauma exposure.  In our study of cross-sectional nature, longitudinal basal cortisol time points were not available, and therefore earlier-life profiles still require elucidation.

Adding further to the complexity of the process of characterisation of HPA-axis anomalies, different subtypes of maltreatment as well as synergetic effects across the different forms of abuse and neglect may present with different profiles. Research suggests that different subtypes of abuse and neglect may have different or even opposite effects on HPA-axis responsivity when assessed in adulthood (Flory, et al., 2009; Schafer, et al., 2010). Accordingly, it has been proposed that sexual abuse and sexual abuse combined with physical abuse, may produce hypercortisolism, whereas physical abuse alone may lead to hypocortisolism (Cicchetti & Rogosch, 2001). Table 2.1 compares both basal and reactive HPA-axis activity, in children as well as adults, on the backdrop of childhood trauma, including different subtypes of trauma, with two studies taking levels of anxiety into account.

As evident from the table, very limited research has been conducted on HPA axis (re)activity in combination with anxiety, in the absence of an anxiety disorder, and to our knowledge, no literature is available pertaining to trauma-mediated HPA-axis perturbations in relation to anxiety, excluding anxiety disorder diagnosis.

Nevertheless, in summary, children with histories of physical and sexual abuse tend to exhibit decreased diurnal variation of cortisol levels, as did adults with histories of severe childhood maltreatment. With the exception of one study in adults previously exposed to childhood sexual abuse, which shows increased HPA-axis reactivity in response to a psychosocial stress test, trauma, in general, seems to be associated with decreased HPA-axis responsivity in adulthood.  Finally, pre-adolescent children with high levels of anxiety exhibit increased morning cortisol levels, which is in contrast to that of children and adults who have been exposed to trauma in early life, but whose anxiety statuses are not known. Since the latter research has been performed in children who did not experience trauma, this finding could point towards differential effects of anxiety *versus* trauma on HPA axis function.

**Table 2.1.** Comparison of studies assessing basal and/or reactive HPA-axis function

| Study | Age group | Type of trauma | Basal [cortisol] | Dex test | Psycho-social stress test | [Cortisol] post challenge | Anxiety |
|---|---|---|---|---|---|---|---|
| (Elzinga, et al., 2008) | Adults | ↑ no of adverse events | | | X | ↓ | |
| (Carpenter, et al., 2007) | Adults | Childhood trauma | | Dex/CRH test | X | ↓ cortisol; ↓ ACTH | |
| (Cicchetti & Rogosch, 2001) | Children | Histories of physical and sexual abuse | ↓ morning cortisol (trend); ↓diurnal variation | | | | |
| (Cicchetti, et al., 2010) | Children | Early physical & sexual abuse compared to early neglect & emotional abuse | atypical flattening of [cortisol] over daytime hours | | | | |
| (Saltzman, et al., 2005) | Children | Marital violence | rise in [cortisol] from morning to afternoon | | | | |
| (Carpenter, et al., 2011) | Adults | Total CTQ (trauma) score | | | X | No relationship | |
| (Watson, et al., 2007) (Carpenter, et al., 2011; Carpenter, et al., 2009) | Adults | Childhood emotional neglect, emotional abuse, physical abuse | | | X | ↓ | |
| (Carpenter, et al., 2007) | Adults | Childhood sexual abuse | | | X | ↑ | |
| (Stein, 1997) (Newport, et al., 2004) | Adults | Childhood sexual abuse | | X | | ↓ | |
| (De Bellis, et al., 1994; Heim, et al., 2001) | Girls and woman | Childhood sexual abuse | | CRH test | | ↓ACTH | |
| (van der Vegt, et al., 2010) | Adults | Severe childhood maltreatment (modifying relationship btw cortisol and anxiety) | ↓daily [cortisol] (4 measures) | | | | Internalising (anxiety) disorders |
| (Greaves-Lord, et al., 2007) | Pre-adolescents | | ↑Morning [cortisol]; awakening response [cortisol] | | | | Persistent anxiety problems |
| (MacMillan, et al., 2009) | Adolescents | Childhood maltreatment | | | X | ↓[Cortisol] | |
| (Ouellet-Morin, et al., 2011) | Pre-adolescents | Childhood adverse experiences | | | X | ↓[Cortisol] | |
| (Greaves-Lord, 2007) | Pre-adolescents | ↑parental internalizing problems (↑familial vulnerability) * | ↑Morning [cortisol] | | | | Anxiety proneness |

* This is not a type of trauma but instead denotes a combination of interview data with both biological parents as well as a genetic aspect, using the following regression equation: genetic risk for internalizing disorder = 0.54 (depression mother + depression father) + 0.43 (anxiety mother + anxiety father).

Accordingly, in the case of long-term maltreatment independent of PTSD, it has been proposed that like the typical HPA-profiles seen in PTSD, a condition of hypocortisolism exists, specifically hyporeactivity to more acute stressors (Fries, et al., 2005; Sherin & Nemeroff, 2011). It has been hypothesized that the duration of maltreatment determines the extent of HPA-axis aberrations (De Bellis, et al., 1999b), and that a trajectory of initial hyperactivation of the HPA-system in childhood, progresses toward a state of chronic adrenal hyporeactivity in adulthood as a type of compensatory adaptation in response to acute hyperexposure to ACTH during stressful early developmental periods (Fries, et al., 2005; Miller, et al., 2007; Pryce, et al., 2005). This

scenario may be due to an eventual adaptive response to the initial prolonged hypercortisolism in more acute cases of childhood maltreatment (Hellhammer & Wade, 1993). This then ultimately results in down-regulation of CRH production in the hypothalamus with up-regulation of its expression in extra-hypothalamic regions, mostly in CRH-producing neurons in the central nucleus of the amygdala (Rosen & Schulkin, 1998), as well as down-regulation of pituitary CRH receptors (Yehuda, 2006). Under these conditions, a scenario exists where HPA-reactivity is repressed, resulting in normal to low cortisol levels under conditions of chronic stress and even in the face of acute psychosocial stressors (Carpenter, et al., 2011; Lee, et al., 2005). In the following paragraphs, with evidence form the literature, we therefore propose a model of initial HPA axis hyperactivity to maltreatment in early life, followed by maladaptation of the axis in the form of lower HPA-axis hyporeactivity during subsequent years all the way through into adulthood, which is modified by anxiety and related modulating modalities such as attachment schemas.

### 2.5.3 HPA-axis maladaptation: a trajectory

In context of the normative progression of HPA-axis changes with age (Kudielka, et al., 2009) which may be a steady increase of cortisol throughout development from middle childhood into early adulthood (Trickett, et al., 2010), it is also important to take into account the onset of maltreatment relative to the age of assessment when investigating HPA-functioning. The developmental stages of childhood as well as puberty have been identified as sensitive periods to experiences of threat and lack of support and signify critical periods where small or transient environmental insults may have disproportionate effects on the subsequent function of biological systems (Doom & Gunnar, 2013; Neigh, et al., 2009). Accordingly, a longitudinal project involving sexually abused girls revealed a state of initial relative hypercortisolism around the age of 11 (DeBellis, et al., 1994), which progressed towards a state of relative hypocortisolism at around age 18, with significantly lower levels of cortisol by early adulthood (Trickett, et al., 2010).

Focusing on basal cortisol levels, a prospective study conducted over the course of 18 years assessed 186 females who have been sexually abused, with morning cortisol levels measured at six different assessments spanning childhood, adolescence, and young adulthood (Trickett, et al., 2010). The linear trend of cortisol measures for the abused females compared to their non-abused counterparts was significantly less steep across development from age 6 to age 30, and cortisol measurements indicated attenuation in cortisol activity starting in adolescence, with significantly lower basal levels of cortisol by early adulthood. More studies support this

notion of past sexual and physical abuse, with blunted diurnal variation of cortisol among abused women (Brewer-Smyth, et al., 2008; Weissbecker, et al., 2006).

However, pertaining to HPA-axis reactivity, in a sample of adult woman, a regression analysis showed that the number of childhood abuse events before puberty had a significant negative effect on peak ACTH concentrations post-TSST challenge, compared to the number of traumatic events during or after puberty, which had a significant positive effect (Heim, et al., 2002). These data support the theory of initial hyperreactivity of the HPA-axis, followed by relative hyporeactivity (i.e. the event of puberty itself is less relevant than the time of assessment relative to time lapsed since trauma exposure). Nevertheless, hypocortisolism in adulthood on the backdrop of childhood abuse has been thought to represent an adrenal gland adaptation to an exaggerated response to stress at the level of the pituitary and/or the hypothalamus (CRH), albeit based on one study where previously abused women exhibit reduced basal plasma cortisol concentrations and produce less cortisol but exhibited an exaggerated ACTH response to both CRH and psychosocial challenge, compared to women without a history of childhood abuse (Raison & Miller, 2003). Despite the fact that we could not find other studies to further support this notion, in our opinion this is an acceptable hypothesis, as it was supported by a recent study in mice involving chronic stress and acute heterotypic stressor-induced corticosterone and ACTH release (Füchsl, et al., 2013).

It still remains to be elucidated whether the above HPA-perturbations reflect adaptations/maladjustments to maltreatment, or pre-exiting protective/risk factors (Luecken, et al., 2009). According to the stress inoculation hypothesis (Eysenck, 1983), exposure to moderately adverse life events may be associated with diminished stress reactivity which may result in resilience in some individuals (De Bellis, 2001; Elzinga, et al., 2008; Garmezy, 1991; Rutter, 1993; Tarullo & Gunnar, 2006). Animal-based research has shown that early life stress (ELS) can serve as resilience in the form of reduced fearfulness and neurobiological reactivity to later stressors (Parker, et al., 2006). Discriminating between ELS that produces vulnerability and ELS that produces resilience thus seems to depend on its severity: in a group of 10-12 year old children, severe but not moderate ELS was associated with heightened sympathetic tone and diminished HPA-activity, which the authors attributed to either a consequence or a cause of resilience (Gunnar, et al., 2009). When studying resilience with regard to HPA-axis reactivity, the context of the stressor such as ongoing family violence as well as the availability of internal and external coping resources must be considered (Hostinar, et al., 2013). Furthermore, early adaptive responses of

hypervigilance to an unpredictable home environment may be maladaptive in other settings, increasing vulnerability for behavioural, emotional and social difficulties (McCrory, et al., 2012).

The different outcomes of hypo- *versus* hyperactivity of the HPA-axis associated with different time points on the trajectory of ongoing childhood maltreatment as well as different types and severity of maltreatment may be modulated by attachment from childhood into adulthood. Adult disorganized or unresolved attachment has been related to maltreatment, specifically to physical abuse and neglect in childhood (Moran, et al., 2008; Neufeld Bailey, et al., 2007; Stovall-McClough, et al., 2008). The Internal Woking Model (IWM) of Unresolved attachment in adults, with respect to experiences of loss or abuse, presented suppressed cortisol reactivity to the TSST (Pierrehumbert, et al., 2009), while the disorganized/disoriented Strange Situation Procedure in infants and early childhood has been associated with enhanced HPA-axis reactivity (Ahnert, et al., 2004; Spangler & Grossmann, 1993; Vermeer & IJzendoorn, 2006), when compared to secure individuals. This may be due to disorganized infants lacking a behavioural coping strategy regulating their emotions (Spangler & Grossmann, 1993), as a result of the approach—avoidance dilemma, where the caregiver represents simultaneously safety as well as being a source of threat, and the HPA-axis therefore remains highly activated. However, in adulthood, Pierrehumbert's group posits that childhood maltreatment events, especially when they have not been psychologically assimilated, may cause a disconnection during subsequent acute stressors, between the perception of stress and HPA-axis reactivity as a way of coping (Pierrehumbert, et al., 2009). Specific to the study of childhood or adolescent sexual abuse, the quality of the family environment (Fassler, et al., 2005) and of the victim's attachment experiences have been described as important moderating, or mediating factors on psychosocial and psychopathological consequences of sexual abuse (Barker-Collo & Read, 2003; Shapiro & Levendovsky, 1999). It appears then that secure attachment may serve as a buffer against the deleterious effects of maltreatment, be it during early childhood years or in adulthood, with the fortunate establishment of healthy attachment bonds with, for example, a partner or psychotherapist. Naturally, this implies that a lack of secure attachment during childhood may represent a modulating factor in the development of anxiety disorders (Bifulco, et al., 2006) as well as low grade inflammation (Hennessya, et al., 2010) in adulthood, due to childhood maltreatment. Although an investigation of attachment schemas is beyond the scope of the current thesis, we have included a measure of self-esteem as an indirect indication of anxious attachment, seeing that the latter is suggested to be linked to dysfunctional attitudes about self and others, with low self-esteem, in turn, resulting in internalizing symptoms such as anxiety.

Given the known interconnectedness of the endocrine and immune systems as major regulatory systems in the body, it is relevant to consider cross-talk between these systems. This is discussed in the next section, with particular reference to the glucocorticoid receptor.

**2.6 Maladaptive sequalae: Cross-talk between the neuro-endocrine and immune systems**

**2.6.1 Inter-relationships between cortisol, DHEAs, testosterone, and prolactin**

Given the deleterious impact of stress in general and especially chronic stress, on the regulation of various hormones (Ranabir & Reetu, 2011), especially in woman presenting with anxiety (Robbins, 2013), hormonal imbalances associated with anxiety and childhood maltreatment warrants investigation. However, the literature on hormonal interrelationships and imbalances in this specific "early stage" of stress-associated maladaptation is limited. In this overview, the term "hormonal imbalances" are used to describe either lower or higher levels of hormones than are still considered to be within population normal ranges, i.e. excluding those associated with general medical conditions, but which may in the long run lead to undesired physiological outcome. Considering the bidirectional communication between the endocrine- and immune systems (Pignata, et al., 2008), hormonal imbalances can thus also exert deleterious effects on certain immune parameters and *vice versa*. The production of the steroid hormones cortisol, Dehydroepiandrosterone (DHEA) and testosterone depend on cholesterol being converted to pregnenolone, and from there the biosynthesis pathway splits into either that of cortisol synthesis, or testosterone, with DHEA a precursor in the androgen hormone synthesis pathways. We postulate that regulation of the direction of this shunt towards either pathway depends on (mal)adaptation to stress and/or anxiety. A more detailed discussion on these hormones as well as prolactin follows.

**CORTISOL:** Cortisol is a steroid hormone, in the glucocorticoid class of hormones, which binds to two subtypes of receptors, namely, a) the high affinity mineralocorticoid receptor (MR), which also binds aldosterone and is fully occupied at low physiological concentrations of glucocorticoids, and b) the glucocorticoid receptor (GR), which is employed during periods of physical or psychological stress when higher concentrations of glucocorticoids are achieved. During conditions of stress, free cortisol enters the cell by passive diffusion and binds to the intracellular (cytoplasmic) GR which is then translocated to the nucleus after dissociation of the regulatory subunits, followed by receptor dimerization. These events culminate at the level of DNA with binding to glucocorticoid response elements, where these sequences can be either activating, negative (repressive) or composite, depending on the binding of other factors.  When acute stress is considered, with

cortisol levels and function upon binding to the GR taking place within the time span of 24 hours, cortisol's circadian rhythm, its rapid, early delayed, and late delayed negative feedback patterns as well as its permissive *versus* suppressive actions on the immune system need to be taken into account. The latter paradoxical permissive and suppressive effects that cortisol exerts on the immune system depend on the level of cortisol (during periods of no stress, acute stress and adaptation to chronic stress), and the degree of cortisol's suppressive effects depend on the time scale after acute stress has been introduced. A model has been developed to incorporate this view and implies that the immune system is primed for activity during the normal circadian cycle. However, an increase in cortisol above circadian levels results in an overall effect of dampening immune system responsiveness, with this effect increasing and decreasing over time in an inverted U fashion and is based on cortisol's opposing effects on cytokine and cytokine receptor expression (Munck & Naray Fejes-Toth, 1992).

A sufficient amount of cortisol released in response to acute stress is known to be generally protective (Buckingham, et al., 1996; Munck, et al., 1984) and its function under these conditions is to contain the immune response. Under these conditions, cortisol is therefore regarded as immunosuppressive, especially as far as cell-mediated immunity is concerned. However, the focus of the current thesis is specifically on effects of long term stress exposure, such as childhood maltreatment, when the allostatic load of stress has already taken its toll on the endocrine- and the immune systems by means of maladaptation of these systems. We have covered the action of cortisol in terms of chronic stress and HPA axis function, in section 2.4.5, and will therefore only consider cortisol's effect on the immune systems and its interaction with specific hormones in the following sections.

**DHEAs:** About 90% of the Dehydroepiandrosterone (DHEA) produced is sulphated to its largely inactive circulating sulphate ester, DHEAs. DHEA exerts its effects *via* its sex steroid metabolites, estradiol and testosterone. DHEAs levels are low since birth until adrenarche starts at six to eight years in both boys and girls, at which point circulating concentrations in both plasma and cerebrospinal fluid begin to rise and DHEAs is synthesized and secreted from the zona reticularis layer of the adrenal cortex (Havelock, et al., 2004; Parker & Odell, 1980). During adulthood, DHEA and DHEAs are released from the zona reticularis of the adrenal cortex and DHEA is also released from the ovary and testis, with circulating concentrations peaking in the mid-20's, after which levels progressively decline with age in both men and women (Maninger, et al., 2008).

DHEA might counter the actions of cortisol (Hu, et al., 2000; Kalimi, et al., 1994) blocking the effects of glucocorticoids on peripheral tissues and the hippocampus (Kaminska, et al., 2000; Kimonides, et al., 1998) as well as exert anxiolytic (Prasad, et al., 1997), antioxidant and anti-inflammatory effects (Chen & Parker, 2004; Russo, et al., 2012), in effect promoting resilience against stress (Pfaua & Russo, 2015). A higher DHEA-to-cortisol ratio has been suggested to be implicated in resilience with regard to the development of or degree of symptom severity in PTSD. For example, DHEA responses to ACTH are elevated in PTSD and negatively correlate with the severity of symptoms (Lipschitz, et al., 2003) and elevated DHEA levels in PTSD have been correlated with symptom improvement and better coping, whereas a lower DHEA-to-cortisol ratio correlates with greater severity of PTSD symptoms (Yehuda, 2006). High cortisol concentrations and low DHEAs concentrations contribute to increases in allostatic load score (McEwen, 2004).

Both DHEA and DHEAs supplementation *in vivo* in rats and mice (Kimura, et al., 1998; Nieschlag, et al., 1973) and *in vitro* in humans (Iwasaki, et al., 2004; Straub, et al., 1998) have been found to decrease activity of nuclear factor-kappa beta (NF-kB), and decreases production of pro-inflammatory cytokines such as TNF-and IL-6.

DHEAs has significant effects on gamma-aminobutyric acid (GABA$_A$) receptor activity and these effects could be involved in the relationship between DHEAs and anxiety disorders (Eser, et al., 2006; Markianos, et al., 2007; Melchior & Ritzmann, 1994) With perhaps the odd exception of low circulating levels of DHEAs observed in individuals with PTSD (Kanter, et al., 2001; Mulchahey, et al., 2001), studies have uniformly identified elevated DHEA and/or DHEAs concentrations in PTSD, as well as increases in the DHEAs-to-cortisol ratio (Bremner, et al., 2007; Butterfield, et al., 2005; Pico-Alfonso, et al., 2004; Rasmusson, et al., 2006; Rasmusson, et al., 2004; Spivak, et al., 2000; Yehuda, 2006). Researchers have suggested that the increase in DHEAs is salutary rather than pathophysiologic (Maninger, et al., 2008) based on the findings of higher DHEAs concentrations associated with fewer PTSD symptoms (Rasmusson, et al., 2004), symptom improvement and better coping (Yehuda, 2006), as well as a decrease in PTSD symptoms in response to psychotherapy (Olff, et al., 2007). In addition to the consistent notion that higher endogenous DHEA concentrations in PTSD patients are related to enhanced resilience,  (Charney, 2004; Yehuda, et al., 2006a) this premise seem to also hold in severely stressed individuals such as veterans, presenting with fewer symptoms of dissociation and superior military performance (Morgan, et al., 2004; Taylor & Liberzon, 2007).

**PROLACTIN:** Prolactin is a polypeptide hormone that is synthesized and secreted from lactotrophs in the anterior pituitary gland, as well as synthesized within the central nervous system and the immune system. Prolactin is synthesized by immune cells and immune cells express its receptor (Berczi, 1994; Gala, 1990; Kelly, et al., 1991; Weigent, 1996). Research in animals has led to the conceptualization of prolactin as a "stress hormone" since serum prolactin levels significantly rise upon introduction of acute stressors and this stress related secretion is inversely related to alterations in stress induced glucocorticoid release (Taylor, et al., 1995). A significant positive correlation has also been observed between day-to-day fluctuations in plasma prolactin and self-perceived anxiety in men confined on a boat for 14 days (Jeffcoate, et al., 1985). Immediate anticipation of an examination has been found to increases plasma prolactin levels and this increase was proportional to the amount of anxiety provoked, as evaluated by the STAI state anxiety version. More specifically, higher anxiety and prolactin levels were associated with the anticipation of a physiology exam, when compare to a psychology exam in medical students, suggesting that different levels of prolactin can be used to discriminate between different intensities of acute stressors (Armario, et al., 1995). However, there are gaps in the more recent literature pertaining to prolactin as a marker for anxiety and chronic stress.

In terms of its effect on the immune system, prolactin plays a significant role in regulation of the humoral and cellular immune responses in physiological as well as pathological states, such as autoimmune diseases (Buskila & Shoenfeld, 1996; Neidhart, 1998; Walker, et al., 1993). Prolactin has been found to enhance immune system function and given cortisol's general immunosuppressive action, prolactin may play a counter-regulatory role to that of cortisol with regard to immune system function (Berczi, et al., 1996). Indeed, in a rat model, prolactin release induced by 60 minutes of psychological (restraint) stress over a period of 10 days, has been found to increase intestinal inflammation by altering the properties of dendritic cells to produce IL-6 and IL-23, both *in vivo* and *in vitro*, which, in turn, played a critical role in altering regulatory T cell phenotypes (Wu, et al., 2014). *In vitro*, prolactin has been found to enhance cell-mediated cytotoxicity and is regarded pro-inflammatory in nature (Berczi, 1993).

**TESTOSTERONE:** The androgen steroid testosterone, the end product of the hypothalamic-pituitary-gonadal (HPG) axis, down-regulates the integrated stress response and has emerged as a potential pro-resilience factor in men (Russo, et al., 2012). Testosterone exerts its effects at multiple levels, including the amygdala and the bed nucleus of the stria terminalis, and along the different nodes of the hypothalamic-pituitary-adrenal (HPA) axis,

possibly by acting upon central neuropeptidergic pathways that control CRH and arginine vasopressin expression (Hermans, et al., 2007b).

Lower levels of salivary testosterone and testosterone levels with less diurnal fluctuation were related to higher levels of anxiety in adolescent boys (Granger, et al., 2003). A role for the amygdala and the effects of testosterone on emotional processing has been suggested. Firstly, by administration of testosterone, cardiac accelerative responses (van Honk & De Haan, 2001) and amygdala activation (Hermans, et al., 2004) were induced to angry facial expressions in healthy volunteers. Secondly, vigilant attentional responses to angry faces related positively to levels of testosterone in males as well as in females (van Honk, et al., 1999). In addition, given its anxiolytic (Suarez-Jimenez, et al., 2013) and fear-reducing properties evident in a number of animal models (Aikey, et al., 2002; Bouissou & Vandenheede, 1996; Frye & Seliga, 2001), testosterone may also reduce the emotional response to the fearful facial expressions. Indeed, this has been the case in a study on healthy females, where a single dose of testosterone significantly reduced the habitual vigilant emotional response to the masked fearful faces in the masked emotional Stroop task, although self-reported measures of anxiety remained unaffected. This provides evidence for fear-reducing properties of testosterone in humans and for testosterone's effects on motivation and emotion concerning the subcortical affective pathways of the brain (van Honk, et al., 2005). The downregulation of the integrated stress response by testosterone may also pertain to the autonomic nervous systems since in anxiety-prone young woman, administration of exogenous testosterone subsequently led to a reduced skin conductance response as well as reduced affective startle modulation in response to pictures with strongly aversive, neutral, or positive content, when compared to the placebo control group (Hermans, et al., 2007a). These findings point towards the possible treatment of anxiety with exogenous testosterone – an avenue to our knowledge not yet explored.

Even though cortisol, prolactin, and testosterone are considered to be "classical stress hormones" (Rose, 1984), care should be taken in interpreting cross-sectional measurements of these hormones as indicators of stress/anxiety/resilience. Taken together, the above identified hormones can possibly serve as markers of resilience/vulnerability. However, in order to get a more precise representation of specific hormonal imbalances associated with the profiling of our unique cohort of study participants, these hormones should rather be assessed in relation to each other, as opposed to considering single measurements in isolation. For markers of resilience, we propose higher DHEAs levels and higher levels of testosterone when compared to appropriate controls, whereas hypo-responsiveness of the HPA-axis, accompanied by high levels of prolactin, may be

associated with chronic inflammation and hence vulnerability to the effects of childhood maltreatment and/or anxiety. In addition to investigating the interrelatedness of the above highlighted hormones by considering their respective levels in relation to each other, the extent to which these hormones exert their actions, i.e., the degree of hormone sensitivity at a cellular level, also comes into play. In the next section (2.6.2), we therefore provide an account of specifically the glucocorticoid receptor (GR) as a measure of cortisol signalling, where GR function provides the link between the endocrine and immune systems, by way facilitating cortisol's effects on certain immune parameters.

**2.6.2 Glucocorticoid receptor number and function**

Dysregulation of the HPA-axis in maltreated individuals is multi-faceted: insufficient CRH release, pituitary hyporesponsiveness to CRH, insufficient adrenal glucocorticoid secretion as well as peripheral decrease in GR levels (or reduced binding affinity to its ligand, nuclear translocation, DNA binding or interaction with other transcription factors) contributing to glucocorticoid (GC) resistance, and these all exhibit the potential to create a systemic pro-inflammatory environment (Desmet, et al., 2014; Silverman & Sternberg, 2012). The high degree of variability between studies investigating cortisol release in maltreated individuals with or without anxiety, in addition to the inter- and intra-individual variation of cortisol levels, necessitates consideration of adaptation in GR number and function as a mechanistic model of GC resistance.

In terms of GR number in the periphery, childhood maltreatment - as measured by parental loss, childhood maltreatment, and parental care - in healthy adults, has been associated with increased NR3C1 promoter methylation of total leukocyte GR, denoting decreasing transcription of the receptor, and this was in turn linked to attenuated cortisol responses to the Dex/CRH test (Tyrka, et al., 2012). In combat related PTSD patients, compared to healthy controls, subpopulation specific intracellular GR analysis yielded the following: 1) PTSD patients had a lower relative quantity of GR in all lymphocyte populations assessed, 2) NK cells of both groups showed higher expression of GR than other lymphocyte subsets, and 3) in PTSD patients, the expression of GR in B lymphocytes was also higher than in T cells (Gotovac, et al., 2002). In addition to GR number, the synergistic actions of neuroendocrine-immune disturbances due to chronic psychological and immune stressors can further contribute to the development of glucocorticoid resistance *via* impaired GR function, (Barden, 2004; Zunszain, et al., 2011). In fact, many clinical features, such as continued state of sickness/depressive behaviours, lethargy/fatigue, reduced locomotor activity and anorexia, metabolic alterations favouring a sustained catabolic state, muscle wasting, cachectic obesity, insulin resistance, osteopenia and anaemia (Straub, et al., 2010) have

been traditionally attributed to elevated glucocorticoids and could in fact result from impaired GR function, and hence glucocorticoid resistance and ensuing enhanced inflammation (Raison & Miller, 2003).

The GR exerts anti-inflammatory effects by means of GR protein-protein interactions and GR-DNA binding–dependent mechanisms. It  is postulated that in contrast to GR-DNA binding–dependent mechanisms, GR protein–protein interactions are not sufficient for glucocorticoids to exert their full anti-inflammatory effects (Reichardt, et al., 1998), and the functional status of the GR may play a role in the final common pathway in determining relative vulnerability *versus* resilience to many inflammatory-related conditions (Silverman & Sternberg, 2012). However, the reliance of GR's anti-inflammatory effects on GR-DNA binding–dependent mechanisms vary according to differences in  experimental *in vivo* immune challenges employed (Kleiman, et al., 2012; Reichardt, et al., 2001; Tuckermann, et al., 2007) and perhaps these models are not directly applicable in the context of psychological stress serving as immune challenge (as opposed to, for example, lipopolysaccharide (LPS) stimulation), and more relevant (*in vivo*) models are required to fully investigate and understand this complexity.

Relatively recently, a study on women who have been raised in unfavourable socio-economic circumstances provided evidence for functional resistance to GR-mediated signalling by way of down-regulation of genes with response elements for GR in PBMCs (Miller, et al., 2009b). Similarly, another study investigating leukocyte GR expression and binding characteristics compared these between veterans with PTSD, traumatized veterans without PTSD and healthy controls (De Kloet, et al., 2007). This was done by determining the effect of Dex on *in vitro* cytokine release, pre- and post-LPS stimulation, as well as on T-cell proliferation. Findings highlighted that trauma exposure alone was sufficient to decrease GR expression and LPS-induced interleukin-10 secretion in the presence of Dex in the veteran populations, whereas resistance of T-cell proliferation to Dex only occurred in presence of PTSD. Accordingly, it appears then that trauma exposure may be uniquely associated with cell-mediated immunity (*via* decreased cortisol signalling and less stimulated anti-inflammatory cytokine release) whereas the condition of PTSD (extreme levels of anxiety) is associated with a dampened proliferation of T cells in response to cortisol. However, since war and combat-associated trauma is relatively shorter in duration and occurs much later in development when compared to childhood maltreatment trauma, it is difficult to directly extrapolate these findings to our population of interest.

Given cortisol's actions on the immune system *via* the GR, it is necessary to also consider the repercussions of maladaptations of the HPA-axis, on the immune system. In the next sections, we therefore provide literature on the immune system in relation to the endocrine system, and in the context of anxiety and childhood trauma.

**2.6.3 Immune system profiling in anxiety and childhood maltreatment**

Given the interrelated nature of the immune and psychoneuroendocrine systems, the immune system has been suggested to be involved in both risk and resilience in PTSD (Baker, et al., 2012), However, whether this is also true for less severe levels of anxiety not reaching clinically relevant status, remains to be elucidated. In general, with chronic stress, immune system activity is influenced by repeated activation of the sympathetic nervous system. Almost all white blood cells, with the exception of Th2 lymphocytes, express β adrenergic receptors. Under stressful situations, the SAM pathway is activated and adrenergic receptors on these cells (among others) bind epinephrine and norepinephrine, inducing the transcription of cytokines controlled by the transcription factor NF-κB (Bierhaus, et al., 2003; Boers, et al., 1992; van Gool, et al., 1990).

In general, two routes by which cytokines signal to the brain have been proposed: 1) the humoral route followed during immune system activation, which results in high levels of cytokines secreted into the circulation and 2) the neural route followed when cytokines are released locally in tissues displaying vagal innervation, with both routes operating simultaneously or sequentially during immune responses (Besedovsky & Rey, 2007; Roth & De Souza, 2001)). Furthermore, parasympathetic tone inhibits pro-inflammatory cytokine release, which, in turn, is under tonic inhibitory control by the vagus nerve (Thayer, 2009; Tracey, 2002). Normal regulation of neuropsychiatric functioning -and homeostasis in general- is dependent on the balance between pro-inflammatory and anti-inflammatory cytokines (Loftis, et al., 2010). However, it was suggested that under conditions of chronic psychosocial stress, parasympathetic nervous system and vagal activity is compromised, resulting in less inhibition of cell-mediated (Th1) cytokines (Thayer & Sternberg, 2010; Tracey, 2002) and a shift from Th2 to Th1 cellular immune responses (Reiche, et al., 2004).

Mast cells, in particular, have been implicated as major role player in the response to psychological stress, when neuropeptides such as brain derived Substance P, CRH and mast cell mediators such as serotonin and tryptase are released form sensory nerves, activating mast and other inflammatory cells (van der Kleij & Bienenstock, 2005). At the level of the brain, hypothalamic mast cell activation leads to stimulation of the HPA axis, for example by regulating CRH release *via* the action of histamine (Cromlish, et al., 1987) or by releasing CRH themselves (Kempuraj, et al., 2004).  This response suggests mast cell involvement in HPA hyperreactivity that

seems to precede the maladaptive hyporeactivity observed in anxiety, mentioned throughout the current thesis. This cell type thus seems an obvious target in the development of anxiolytic treatment *via* immune modulation.

In addition to the steadily emerging body of research pointing towards a pro-inflammatory phenotype associated with 'affective spectrum disorders' that include anxiety, major depressive disorder, fibromyalgia, irritable bowel syndrome and chronic fatigue syndrome (Hou & Baldwin, 2012), childhood trauma has also been associated with the development of certain immune diseases later in life, probably by potentiating a shift towards a pro-inflammatory state (Danese, et al., 2007).  In the extreme case of trauma exposure resulting in PTSD, women with PTSD related to childhood sexual and physical abuse demonstrated enhanced delayed type hypersensitivity, a physiological *in vivo* measure of cell-mediated immunity (Altermus, et al., 2003). This shift in the developmental trajectory towards a pro-inflammatory phenotype has been shown to result from even mild exposure to a risky family in early life, with a 10% increase in stimulated IL-6 release for every half-point increment in harshness on a 5-point continuum and a 4% decline *in vitro* sensitivity to cortisol in adolescent girls aged 15 to 18 years (Miller & Chen, 2010). Furthermore, over a period of 1.5 years, this study found increased *in vitro* IL-6 release and decreased sensitisation of GR in monocytes post-endotoxin challenge as well as in response to a psychosocial stress test in woman raised in harsh family climates, as assessed by the Risky Families Questionnaire (Taylor, Eisenberger, Saxbe, Lehman, & Lieberman, 2006).

In a prospective birth cohort study, after controlling for current stress (at age 20), childhood maltreatment was estimated to contribute to more than 10% of low-grade inflammation when using a C-reactive protein (CRP) measure in adulthood (Danese, et al., 2007). Similarly, associations have been found between childhood/adolescent sexual abuse and higher basal levels of IL-6 and CRP (Bertone-Johnson, et al., 2012) as well as between childhood adversity and heightened IL-6 and TNF-α levels in later life, when compared to controls (Kiecolt-Glaser, et al., 2011). In a prospective study,  childhood (6 – 8 years) adverse events as well as cumulative adversity from birth to 8 years were associated with higher levels of IL-6 and CRP at age 10 and predicted increased levels of CRP at age 15 (Slopen, et al., 2013).

Evidence for an acquired pro-inflammatory phenotype exists also in the context of anxiety and involves enhanced immune system activation in the form of elevated serum levels of CRP, Tumour Necrosis factor-α (TNF-α), interleukin-1β and its receptor (IL-1βR), IL-6, IL6-R and IL-8,  which have been reported for both anxiety and PTSD (Black, 2002; Gill, et al., 2009; Hoge, et al., 2009; Ironson, et al., 1997; Pace & Heim, 2011; Pervanidou, 2008; Solomon, et al., 1997; Spivak, et al., 1997; Von Kanel, et al., 2010). However, as is the case

with studies focused on HPA-axis function, to our knowledge, no study has investigated immune function in relation to childhood trauma and anxiety simultaneously and in the absence of PTSD diagnosis. Therefore, in the next section, we provide an overview of the literature on trauma-induced progression towards a pro-inflammatory phenotype, which has been studied more comprehensively, with some authors also considering the relevance of this occurrence in PTSD patients.

**2.6.4 Immune system malfunction: Another trajectory**

The developmental period of roughly 18 to 25 years has been conceptualized as a time when individual trajectories of health become more firmly established and signifies a period of increasing risk for the development of psychopathology (Arnett, 2000; Arnett, 2007; Masten, et al., 2004; Romer & Walker, 2007). Strikingly, early life adversities do not even have to be severe in order to have a lasting physiological impact as, even after controlling for self-reported sexual or physical abuse, as well as above average experiences of abuse, a study has found attenuated cortisol in those exposed to childhood families characterized as high in conflict, low in cohesion, and low in expressiveness when compared to participants reporting more positive early family relationships (Luecken, et al., 2009). In addition to the afore mentioned low-grade inflammatory profile evident during the ensuing years of childhood maltreatment, is the hypothesis that the immune response is also primed to over-react to new stressors, be it pathogenic or psychosocial in nature. For example, healthy subjects who have experienced childhood maltreatment exhibited greater acute IL-6 release and higher IL-6 concentrations over time during the course of the TSST, when compared to a non-maltreated group (Carpenter, et al., 2010). Furthermore, considering a more "end-stage" scenario pertaining to trauma, anxiety, and a primed immune system in a recent study, consisting of severely affected patients with war and torture experiences, mainly experienced during late adolescence and adulthood and with a chronic disease pattern (compared to 25 healthy ethnically matched control subjects): results included significantly increased spontaneous production of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in PBMCs in the PTSD group, which may point towards pre-activated PBMCs in the PTSD patients (Gola, et al., 2013).

The above discussed pro-inflammatory phenotype as well as exaggerated immune responses to stressors observed in  individuals who have experienced years of childhood maltreatment - abuse and neglect, trauma, early life stress, maltreatment and/or adverse or risky family  lives-, have been suggested to be a consequence of 1) exposure of the still developing immune an endocrine systems  to these conditions during early sensitive periods 2) monocytes/macrophages being programmed to respond with tendencies that give rise to a chronic

pro-inflammatory state, and 3) activation of the innate immune system  which has, in turn, a spin-off on the adaptive immune system where B lymphocyte function may be supressed and T lymphocyte activity is predominantly governed by Th1 cellular immunity and pro-inflammatory release (Coelho, et al., 2014; Miller, et al., 2011). An illustration as to how immune cells may be susceptible to early imprinting at the level of DNA, which may be linked to psychological status, is the report of accelerated leukocyte telomere shortening in patients diagnosed with an internalizing anxiety disorder, particularly in the first half of the life course (Shalev, et al., 2014). We propose a relationship between psychopathology and the immune system where long lasting imprints as a consequence of childhood maltreatment are made on the immune system during early sensitive periods, which together with hormonal variables may predispose an individual to the development of an anxiety disorder.

A question that begs further investigation pertains to how these immunological aberrations would manifest clinically, providing a distinct "fingerprint" of maltreatment. A survey of a large sample of 11 367 participants indicated that a history of childhood trauma was associated with higher lymphocyte counts later in adulthood (Surtees, et al., 2003), and it has been postulated that systematic differences in the total white cell count might be more likely to occur when individuals also differ in their neuroendocrine activity (Lemieux, et al., 2008). A meta-analysis revealed that as the number of adverse childhood experiences increased, the likelihood of hospitalizations with Th1, Th2, Th2-rheumatic, and any of 21 types of autoimmune diseases also increased for decades into adulthood (Dube, et al., 2009). Already in childhood and adolescence (up to the age of 18), heightened levels of pro-inflammatory cytokines have been repeatedly observed in those with psychiatric disorders, according to a recent systematic review, albeit not pertaining to childhood maltreatment and anxiety *per se* (Mitchell & Goldstein, 2014).

However, the fold-change in levels of pro-inflammatory cytokines in blood from psychiatric patients with histories of childhood maltreatment do not seem to be of the same magnitude as those in the blood of inflammatory-related disorders patients such as rheumatoid arthritis (Chen, et al., 2009; Dean, 2011). These profiles may also vary somewhat between woman and men in general as well as in the context of autoimmune diseases, since the basic immune response differ between males and females. For example: 1) women respond to infection, immunization, or trauma with higher antibody production whereas inflammation is usually more severe in men (Cutolo, et al., 2006; Fairweather, et al., 2008; Frisancho-Kiss, et al., 2007); 2) glucocorticoids decrease cell-mediated Th1-type immunity in response to acute stress (Beeson, 1994), and oestrogen

transcriptionally up-regulates glucocorticoid levels *via* its effect on CRH, whereas testosterone decreases glucocorticoid levels in males (Cutolo, et al., 2006; Elenkov & Chrousos, 2002; Viau, 2002); 3) oestrogen increases IL-4 levels in females, resulting in a greater Th2-type immune response (Beeson, 1994); 4) testosterone reduces glucocorticoid and IL-4 levels in males, resulting in a predominantly IFN-y associated Th1-type immune response to infection or trauma (Cutolo, et al., 2006); and 5) evidence points to sex prevalence of certain ADs (Fairweather, et al., 2008; Kumar, et al., 2005; Rose & Mackay, 2006).

Different mechanisms may also underlie the discrepancies in different displays of chronically heightened inflammatory activity, through primarily increases in HPA- and sympathetic-activity. Hansel and his group reviewed five models of how chronic stress may induce harmful inflammatory activity; 1) systemic overload with subsequent exhaustion of adrenal glands, autonomous nerve fibres, or hypothalamic-medullar structures resulting in decreased anti-inflammatory feedback; 2) initial increased GR expression followed by GC resistance, also resulting in decreased anti-inflammatory feedback; 3) recurrent infections (secondary inflammation) due to chronically elevated cortisol levels which raise the susceptibility to viral infections, along with decreased antibody production 4) disease-specific alterations of stress-immune interactions, such as in Crohn's disease and asthma and 5) locally harmful effects as a result of pro-inflammatory effects of stress hormones (Hansel, et al., 2010). In addition, pro-inflammatory cytokine production in children with psychiatric disorders (Lilic, et al., 1997), as well as illness duration and load in children differ, when compared to adults (Danese & McEwen, 2012; Kapczinski, et al., 2008; McEwen, 1998), therefore, the different models of inflammation should be interpreted within the context of age. Furthermore, evidence exists for inter-individual differences between cellular *versus* humoral immunity, even within the same psychiatric disorder, based on the predominant constellation of psychological symptoms. As noted in the previous section on HPA-axis function, gaps in the literature pertain also to different immune endophenotypes, in the context of childhood maltreatment and anxiety proneness. Table 2.2 summarises specific immune profiles reported in individuals with or without PTSD diagnosis, associated with specific symptoms and trauma types. However, perhaps, given the different foci of these studies, but also the varied nature of populations assessed, no conclusion can be made from these data, except perhaps to highlight the danger of generalisation of results by extrapolation to other populations.

**Table 2.2** Immune profiles associated with complex trauma

| Study | PTSD | Type of trauma | Symptoms | Effect on the immune system |
|---|---|---|---|---|
| (Woods, et al., 2005) | Yes | Interpersonal violence | 1. Intrusive symptoms<br>2. Avoidance | 1. + association with CD4+ cells, at the expense of CD8+ cells<br>2. + association with both CD4+ and CD8+cells and symptom severity modulating the relationship between an abuse history and the increased production of IFN-γ by T cells |
| (Lemieux, et al., 2008) | Yes | Abuse | Positive correlation with intrusive symptoms; Negative with avoidant symptoms | Independent of cortisol and NE levels: ↑ percentage of CD8+ lymphocytes expressing the early activation marker CD45RA+, when compared to controls and those exposed to abuse but not meeting PTSD criteria |
| (Wilson, et al., 1999) | No | Sexual abuse | | ↑ in the CD45RO/CD45RA ratio in PBMCs, compared to controls, suggesting ↑ lymphocyte immune activation and T cell memory |
| (Altemus, et al., 2003) | Yes | Sexual abuse | | Larger delayed type hypersensitivity responses (DTH) |

**2.6.5 Assessing progression on the pro-inflammatory trajectory**

In individuals suffering from anxiety and/or childhood trauma, the progression towards a pro-inflammatory phenotype can be monitored by assessing the balance between pro- and anti-inflammatory markers, denoting either cell-mediated or humoral immunity. Domination of either type of immunity is signified by, for example, specific white blood cells types and cytokines in circulation. For a comparison between the cell-mediated *versus* humoral immunity, please see Table 2.3 below.

**Table 2.3** A comparison between cell-mediated and humoral immunity

| Determinants | Type 1/ Th1 Cell-mediated (delayed hypersensitivity) | Type 2/ Th2 Humoral (allergens) |
|---|---|---|
| **Effector cells** | 1. B (CD19/20) lymphocytes<br>2. CD3+ Th1 cells<br>3. CD 8+ T cytotoxic (Tc) cells<br>4. Macrophages | 1. B (CD19/20) lymphocytes<br>2. CD4+ Th2 cells<br>3. Mast cells<br>4. Eosinophils |
| **Maturation cytokines** | IL-12 released by activated macrophages and dendritic cells cause differentiation of naïve T0 cell into Th1 cells | IL-4 causes differentiation of naïve T0 cell into Th2 cells |
| **Effects of Th cell activation** | 1.Th1 cells a) mediate activation and proliferation of Tc cells; c) mediate activation, proliferation and maturation of B-cells c) help recruit and activate cells responsible for the innate inflammatory processes (phagocytosis)<br>2. Th1-activated B cells a) aid complement activation and b) opsonisation for phagocytosis<br>3. Th1-activated Tc cells a) target virally (or bacterially) infected cells b) induce the complement system and apoptosis c) produce IL-2 and IFNγ | 1. Th2 cells a) release cytokines inhibiting inflammation; b) cause the differentiation and activation of eosinophils; c) activates B cells<br>2. Th2-activated B cells a) Neutralize pathogens *via* release of IgG1 antibodies and b) release IgE antibodies which cause mast cell degranulation |
| **Cytokines released by Th cells** | IL-2 (IL-2 is produced by all helper T cells early in their activation although Th1 cells continue releasing IL-2 throughout their lifespan); interferon-γ (IFN-γ); Tumour Necrosis Factor – β (TNF-β); IL-12 | IL-4; IL-5; IL-6; IL-10; IL-13 |
| **Effects of cytokines** | 1. IFN-γ increases the production of IL-12 by dendritic cells and macrophages, and *via* positive feedback, IL-12 stimulates the production of IFN-γ in helper T cells, thereby promoting the Th1 profile<br>2. IFN-γ inhibits the production of cytokines such as IL-4, an important cytokine associated with the Type 2 response, and thus it also acts to preserve its own response | 1. Interleukin-4 causes Th2 cells to promote the production of Th2 cytokines (including itself as it is auto-regulatory)<br><br>2. IL-10 inhibits a variety of cytokines including interleukin-2 and IFN-γ in Th1 cells and IL-12 in dendritic cells and macrophages |

We propose that a dominance of Th1 immunity at the expense of Th2 immunity can be established in an individual by firstly considering the distribution of white blood cells in circulation. Normal percentages of the different cell types in peripheral blood include 55% for neutrophils, 35% for lymphocytes, and 6.5% for monocytes. Increases in cortisol levels cause a loss of lymphocytes and mononuclear cells from circulation, whereas neutrophil numbers increase in circulation. Therefore, in the case of (lower) hypoactivity of the HPA-axis, conducive of cellular immunity, low levels of cortisol would result in higher levels of lymphocytes as well

as monocytes. In contrast, high levels of cortisol promote antibody generation by Th2 driven B cell proliferation, thereby increasing B cells while decreasing Th1 cells as well as NK cells. Furthermore, T cells are more sensitive to cortisol suppression than B cells, suggesting another role for cortisol in maintaining a balance between Th1 versus Th2 immune responses. Furthermore, a cell type which has been specifically implicated in inflammation is NKT cells.

NKT cells, constituting about 2% of peripheral blood T cells (Doherty, et al., 1999) are identifiable by using the CD56 and CD3 markers and were first characterized in humans by Lanier *et al*, (Lanier, et al., 1986) and Schmidt *et al* (Schmidt, et al., 1986). This cell line is a population of T cells that share some characteristics with natural killer (NK) cells, characterized by high levels of cytokine production, particularly interleukin 4 (IL-4) and interferon γ (IFN-γ), where cytokine secretion depends on the microenvironment of these cells (Godfrey, et al., 2000). However, NK+ T cell receptor (TCR)+ lymphocytes are heterogeneous and, therefore, the precise definition of an NKT cell remains problematic. Accordingly,  NKT cells have been defined as cells that nearly always have an invariant Vα14-Jα18 rearrangement and recognize lipid antigens in conjunction with the class I–like molecule cluster of differentiation (CD)1d (Godfrey & Kronenberg, 2004).

Humans appear to have a heterogeneous repertoire of NKT cells that can express αβ or γδ TCR and NK cell markers including CD16, CD56, CD69, CD161 (McMahon & Raulet, 2001). Human NKT cells  recognize the glycosphingolipid α-galactosylceramide (αGalCer) presented by the nonclassical major histocompatibility complex (MHC) class I-like molecule CD1, where the CD1d-dependent NKT cells can be broadly divided into two types of cells including type I and type II NKT cells (Gao, et al., 2009). More specifically, four categories of NKT cells have been described: a) Vα14i T cells which are CD1d autoreactive, do not express CD8 and are either CD4 + or double negative - also referred to as Type I or invariant NKT (iNKT) cells; b) NKT cells, also CD1d autoreactive and expressing either CD4 + or DN, but with expression of TCRs that are more diverse - also referred to as Type II or non-iNKT cells, c) NKT cells that are not CD1d dependent, have diverse TCRs and can be CD8 +, CD4 + or DN, and d) NKT cells are defined by the expression of CD49B (α 2 -integrin), which is recognized by the DX5 antibody (Kronenberg & Gapin, 2002).

From the above, it is clear that the NKT population in itself is quite heterogeneous. Relevant to the current topic, involvement of NKT cells in tipping the scale towards either Th1 versus Th2 immunity may rely on the transcription factor T-bet, which plays a crucial role in regulating Th1 cytokine gene expression, as it has been implicated in the expression of genes controlling the migration, survival and effector functions of iNKT cells

(Matsuda, et al., 2006). A shift towards a Th1 profile may also be mediated by specifically *via* activation of the double negative iNKT cells which exhibit a Th1 cytokine production profile (Godfrey, et al., 2004; Seino & Taniguchi, 2005). Thus, in order to draw conclusions on the role of the NKT cells in the context of inflammation, assessment of only total NKT cell populations are insufficient. Rather, an attempt should be made to distinguish the relevant subpopulations as required.

Furthermore, to our knowledge, only one study has aimed to investigate NKT cell function in relation to psychological stress and this was in a rat model (Oya, et al., 2000). Research by Oya and colleagues has revealed a slight increase in liver NKT cell number and a significant self-reactive increase in NKT cytotoxicity in response to 24 hours of restraint stress as well as augmentation of NKT cytotoxicity post *in vivo* administration of adrenalin or hydrocortisone. In addition to assessing the downstream functionality and cytokine secretion pattern of NKT cells in response to stress, NKT function may also be directly linked to the general stress response by means of analysing NKT cell expression of receptor level and/or function associated with both the SAM and the HPA-axis. Although not in the context of chronic psychological stress or anxiety *per say*, NKT cells have been shown to express cell surface adrenergic receptors (Fukuda, et al., 1996; Suzuki, et al., 1997) which increase in number or proportion by sympathetic nerve stimulation (Tsukahara, et al., 1997; Yamamura, et al., 1996). However, given the heterogeneity of the NKT population discussed above, it is impossible to draw firm conclusions from these data. To our knowledge, no study up to date has assessed GR expression in NKT cells, in association with anxiety and/or trauma. This remains an avenue for investigation, but given the complexity of NKT subpopulation marker identification and the fact that this thesis was relatively generally exploratory, NKT cells were not a specific interest here.

Another means of determining whether a shift towards Th1 immunity has taken place, is by measuring levels of circulating pro-inflammatory cytokines. Polarization of the immune response to either that of Th1 or Th2 cytokines, depends on the micro environment of the effector cells, which can either promote a shift towards Th2 function *via* the availability of cortisol and IL-4, or polarization towards Th1 immunity *via* the actions of IL-12. Again, in the case of lower HPA-axis hypoactivity, the relationship between levels of circulating cortisol and pro-inflammatory cytokines need to be taken into consideration. Cortisol's role in the regulation of inflammation is multifold. Firstly, cortisol generally suppresses pro-inflammatory cytokines, specifically IL-1, IL-2, IL-3, IL-6, IL-8, IL-12, and TNF-α, but not the anti-inflammatory cytokine, IL-10.

Considering the pro-inflammatory cytokine IL-12, induction of the bioactive IL-12p70 heterodimer (composed of p35 and p40 subunits) during dendritic cell interaction with either Th1 or Th2 cells depends on the triggering of CD40 by CD40 ligand present on activated Th (1 or 2) cells, but disproportionately high levels pf IL-12p70 production requires an additional co-stimulatory signal *via* IFN-γ, a product of Th1 cells, Tc cells, and NK cells (Kalinski, et al., 2000). In contrast, IL- 12p40 homodimer, secreted by antigen presenting cells in the absence of p35 expression do not mediate Th1 polarization (Kalinski, et al., 2001).

In addition to assessing pro-inflammatory cytokine levels to establish whether there is dominance of Th1 immunity, a measurement of serum levels of Myeloperoxidase (MPO) can also be used to assess levels of systemic inflammation. MPO is a pro-oxidative and pro-inflammatory enzyme stored in azurophilic granules in neutrophils and monocytes and released into extracellular fluid in the setting of inflammatory processes. MPO may contribute to the chronic, non-microbial inflammatory process in various diseases. For example, compared to controls, serum MPO levels were higher in patients with chronic inflammation (rheumatoid arthritis, systemic lupus erythematosus, primary Sjögren's syndrome, dermatomyositis  and ankylosing spondylitis) (Wang, et al., 2014).

Taken the above sections constituting Part B into consideration in terms of contribution to the precision medicine paradigm, we highlighted gaps in the literature pertaining to the simultaneous investigation of childhood trauma and anxiety in relation to neurophysiological maladaptations, in adolescence. In the context of hypoactivation of the lower HPA-axis, we highlighted the following measurable markers of risk: high serum prolactin levels, low serum cortisol and DHEAs levels, high GR expression, and high levels of pro-inflammatory markers.

## 2.7. Summary

The globally escalating incidence of anxiety disorders necessitating appropriate intervention strategies, along with the notion that prevention is better than cure, provide a niche for precision medicine. In the context of childhood trauma and anxiety proneness - two main role players contributing to the risk of developing an anxiety disorder- the debate surrounding nature *versus* nurture no longer revolves around which is mediating outcome measures associated with pathology. Thanks to discoveries in epigenetics, the mutual contributions of and interaction between nature and nurture can no longer be denied. However, in identifying specific risk factors that are deemed less stable over time due to the modification of genes by the environment, the potential for intervention to modify these factors is increased.

Our model of anxiety posits that anxiety arises due to feelings of uncontrollability, based on a specific constellation of beliefs about the self as well as the self in relation to the external world. Seeing that the extent to which individuals can exercise control over environmental factors, specifically maltreatment, is limited, and that anxiety is a product of the perception of uncontrollability associated with these environmental factors, we suggest that intervention focus on changing these perceptions of reality, rather than trying to intervene at the level of reality itself. However, before such attempts can be made, different psycho-neurophysiological phenotypes associated with different intercepts - depending on the extent of maltreatment, pre-existing resilience/risk factors and age group - on the continuum of the paradigm of anxiety should be investigated to further our understanding in this context. Such a proposed continuum would then range from psychological and physiological homeostasis to psychopathology with accompanying aberrations in neurophysiology. Following this approach will allow firstly for psychotherapy to be tailor-made by way of addressing maladaptive information processing underlying the cognitive, affective and behavioural aspects of anxiety, once specific neural pathways that are subject to modification have been identified. Secondly, by identifying specific neurophysiological profiles associated with anxiety, these could be used to discern whether intervention should involve a) psychotherapy, with the identified physiological parameters used as a diagnostic and/or monitoring tool, or b) medication , where these same neurophysiological measures can be used to facilitate the prescription of tailor made alternative or conventional medicine. In the current study, we aim to contribute to the delineation of the specific neuro-physiological phenotypes, comprising either risk or resilience, in association with anxiety proneness, on the backdrop of a history of childhood maltreatment, in an adolescent population.

**3. Hypothesis and Aims**

We hypothesised that:

1.  Peripherally, high anxiety proneness will correlate with

a) hypoactivity of the lower HPA-axis

b) a pro-inflammatory profile potentiating cell-mediated immunity, while inversely affecting the adaptive immune response

2. Centrally, high anxiety proneness in adolescents will correlate with

a) poorer executive functioning

b) larger right hemisphere volumes

3. In low anxiety prone adolescents, markers potentially associated with resilience, i.e.,  high stress coping ability (resilience) and high measures of specific personality traits such as self-esteem and mixed handedness (reflecting potential cognitive flexibility) will modulate the peripheral endocrine- and immune- systems in a favourable manner

4. High anxiety-prone individuals will have the largest HPA-axis suppression in response to Betamethasone administration and will present with hyporeactivity of the HPA-axis, subsequent to a psychosocial stress challenge

5. Trauma will modulate 1, 2, 3 and 4, by exacerbating parameters associated with risk in the aetiology of anxiety

6. Useful psychological and biological markers associated with both risk and resilience will be identified

In order to investigate these hypotheses, our primary aim was to characterise the central and peripheral neuroendocrine and immunological profiles in older adolescents, in relation to anxiety proneness *versus* childhood trauma, using a cross-sectional study design. A second aim was to assess the potential modulating influences of measures of resilience, handedness and self-esteem on clinical parameters assessed in these adolescents. A third aim was to identify useful and practical psychobiological markers associated with both risk and resilience in anxiety, with this knowledge contributing to a precision medicine paradigm for diagnosis and

treatment, in the South African adolescent context.  In achieving this aim, the identified psychological and biological risk factors can a) add to a body of knowledge to be used in future, in the identification of individuals at risk for pathology, and b) indicate how existing treatment strategies in South Africa can be tailored to address these factors.  A fourth aim was to assess whether anxiety proneness or childhood trauma is more closely associated with the identified markers of risk, so as to direct the focus of future research and intervention strategies, appropriately.

In order to achieve our aims, the following specific objectives were formulated:

1. To obtain ethical clearance for our study from the relevant authorities

2. To recruit and conduct a first level screening for the presence of psychological disorders, (based on our exclusion criteria) of randomly selected older adolescents (aged 15-18) from high schools in the Western Cape area

3. To assess the level of childhood maltreatment, including subscales of trauma, as well as anxiety proneness (measures of AS and trait anxiety), for delineation into the four experimental groups, according to level of trauma exposure and anxiety proneness (for statistical purposes)

4.  To conduct a second level screening to confirm group status and measure resilience, self-esteem and handedness *via* administration of appropriate questionnaires

5. To determine a basal HPA-axis hormone profile (cortisol, prolactin, testosterone and DHEAs, in serum) and basal immune status (total white blood cell count, subpopulation specific GR level expression and serum IL-8, IL-1$\beta$, IL-6, IL-10, TNF-$\alpha$, IL-12p70 and MPO)

6. To assess central correlates of anxiety *versus* trauma, i.e., executive function (including working memory), by means of neurocognitive tests, and volumetric differences between the left and right hemispheres of the amygdala, hippocampus, thalamus, and PFC *via* a structural MRI

7. To assess HPA-axis responsiveness and concurrent state anxiety to an *in vivo* Bexamethasone suppression test in conjunction with a psychosocial stress test, by measuring serum and plasma levels of cortisol and ACTH respectively, as well as psychometrically assessing levels of state anxiety.

## CHAPTER 3

## METHODOLOGY

The study was of cross-sectional design and included both descriptive and intervention components. Methodology consisted of five sequential phases, as set out below.

### 3.1 Phase I: Recruitment and first level screening

### 3.1.1 Ethical aspects

This study formed part of a bigger psychiatry-affiliated project, for which ethical clearance was granted by the Stellenbosch University Human Research Ethics Committee (Reference number: N11/04/131). In addition, we obtained permission to access a number of schools in the Cape Town area from the Western Cape Department of Education. Inclusion into the study required written informed assent obtained from the learners, as well as subsequent consent from the parents/legal guardians of the learners, after learners and parents/legal guardians had been informed of the objectives and purposes of the study verbally, with the opportunity to ask questions to ensure complete understanding. Apart from information on the specific purpose of the study, learners were also informed that their participation was voluntary and that they were able to withdraw at any stage, that the information they provided would be kept confidential and that their anonymity would be maintained by coding of the study database and related participant documentation (with only study staff allowed access to participant information). All protocols were carried out in accordance to The Declaration of Helsinki (Edinburgh, 2000) and Medical Research Ethical Guidelines on Human Research Version 2, 2002.

### 3.1.2 Recruitment

1149 Participants were recruited from government schools which were proportionally and randomly selected from the North, South, East, and Central educational districts around Cape Town, in order to provide a representation of the socio-demographic area. Initially, 21 schools were randomly selected and approached to take part in the study in 2011, but subsequent recruitment proved to be tedious:  Departmental approval of the project was only granted at the end of June 2011 and the Western Cape Department of Education did not allow research activities to take place in the 4th term. This left only the 3rd term for recruitment, which was insufficient time for completion of Phase I.

Furthermore, the consent return and screening rates were both very poor – the return rate was only 14% (of the 2660 consent/assent forms handed out to learners, only 372 were signed and returned to the respective schools) and the screening rate was only 9.89% (number of learners screened/number of consent and assent forms handed out). We therefore applied for a shorter, tacit consent and assent form to be used instead, which was approved at the beginning of 2012 (See Appendix A for consent and assent forms).

In 2012, after almost no cooperation from the schools that we were liaising with during the 1st semester, we used the latter versions of consent and assent forms during the 2nd semester, with more success, although not yet sufficient for the purpose of the research.

We therefore randomly selected another 9 schools to approach during 2012. Out of the total of 30 schools (including the initial 21 schools) randomly selected and subsequently approached, 22 headmasters agreed for their learners to participate in the study. The recruitment procedure consisted of obtaining all the class lists from grade 8 to 12, from each school, after which 100 learners (20 per grade) per school were randomly selected. Consent and assent forms were handed out to these selected children at the school and only those participants who returned their signed consent and assent forms, partook in the filling out of questionnaires, at the school. This was done in order for the participants to be pre-screened for eligibility for inclusion and screened for self-reported childhood maltreatment/trauma and anxiety-proneness. Telephone numbers and information pertaining to basic demographics were also obtained from these participants.

### 3.1.3 Screening and selection criteria

The envisaged neurological and physiological parameters were prohibitively expensive, so that it was not feasible to carry out in all recruits. Thus, from the onset of the study, the plan was to reduce the total number of recruits to a representative subgroup for this purpose, while the complete cohort was included for the purpose of descriptive information in the related psychiatry study already mentioned. Thus, the initial cohort of 1149 participants was pre-screened by means of the following questionnaires:

a. The Life Events Timeline **-** participants were instructed to indicate on a timeline at which age/s major life events occurred. Some examples of common life events reported were: "someone close to me died", "fell pregnant", "both parents using drugs", "gang violence in neighborhood", "domestic violence", hospitalized ", "molested by stepbrother", "participant got hijacked", "witnessed father being killed", "hit by a car", "father

abandoned the family", "no food to eat at home", "didn't know father", "attempted suicide", "molested by father", "participant did domestic duties while mother was drinking"; "sibling died in motor vehicle accident"

b. The Center for Epidemiological Studies Depression Scale for children (CES-DC) - a 20-item self-report measure of depression symptoms experienced during the preceding week

c. The Alcohol Use Disorders Identification Test (AUDIT) - a 10-item self-report measure used to identify hazardous and harmful patterns of alcohol consumption in the past year, by assessing recent alcohol use, alcohol dependence symptoms and alcohol-related problems

d. The Drug Use Disorders Identification Test (DUDIT) - an 11-item self-report measure used to identify drug use patterns and various drug-related problems

e. Adolescent Coping Orientation for Problem Experiences (A-COPE) - a 54-item self-report coping inventory used to measure the behaviours and patterns that adolescents find helpful in managing problems or difficult situations

According to the outcome of the above questionnaire assessment, participants were included in terms of the following criteria:

a. Adolescents who were between the ages of 16 and 18 years (our choice of an older adolescent age range was based on possible confounding fluctuations of the physiological parameters as a result of early adolescent (10-14 years) pituitary-gonadal hormonal imbalances (Brooks-Gunn & Warren, 1989; Susman, et al., 1991), which we tried to avoid as far as possible. We were also interested in assessing the more enduring effects of ongoing childhood maltreatment, which in our opinion would be more evident in a slightly older adolescent population.)

b. Adolescents who were able to read, write, and understand English or Afrikaans at 5th grade level

c. Adolescents who were psychotropically naïve

Participants were excluded based on the following criteria:

a. Receiving treatment for anxiety disorders

b. Exhibiting a current history of alcohol or substance abuse or dependence

c. Currently using psychotropic medication

d. Presenting with a history of head trauma

Those meeting the inclusion criteria were matched for age, ethnicity, gender and educational status and screened for self-reported childhood maltreatment/trauma as well as anxiety proneness (a measure of anxiety sensitivity and trait anxiety), using the following 3 questionnaires:

a. The Childhood Trauma Questionnaire (CTQ) - a 28-item retrospective measure of the frequency and severity of abuse and neglect experienced prior to age 18. For the purposes of the current study, we adapted this slightly, to enquire only about maltreatment experienced prior to age 12. The CTQ consists of 5 subscales that assess emotional, physical, and sexual abuse and emotional and physical neglect, respectively.

b. The Child Anxiety Sensitivity Index (CASI) - an 18-item self-report questionnaire that measures the fear of anxiety by asking participants to rate the extent to which they believe the experience of anxiety will result in negative consequences

c. The State-Trait Anxiety Inventory (STAI) - a 40-item self-report questionnaire divided into 2 sections which assess both state and trait anxiety. At this stage of screening, participants were only required to complete the 'trait' section.

Some basic descriptive statistics of the initial cohort of 1149 participants included the following: In terms of age, we report on a minimum of 12, a maximum of 23, and a mean of 16.2 ±1.9. With regard to gender, 40% of the 1149 participants were male and 60% was female. In terms of ethnicity, the majority of the study population was African (Fig 3.1).

**Figure 3.1.** Relative ethnical constitution of the initial cohort of 1149 participants



■ White 1.8%
■ Coloured 27.7%
■ African 68.9%
■ Asian 0.2%
■ Other 1.4%
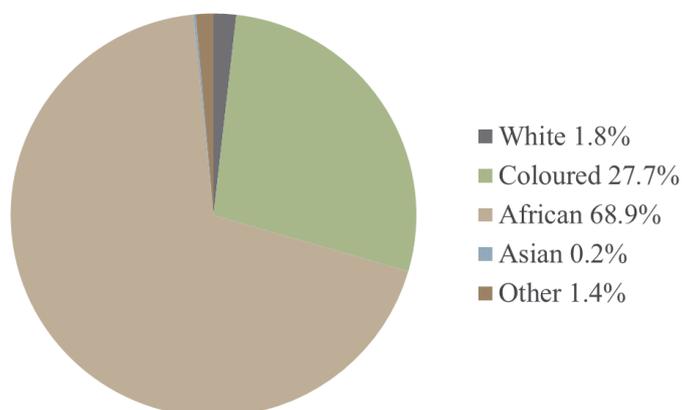
**3.1.4 Delineation of groups**

Four experimental groups (total n = 200) were selected from the initial cohort of 1149, where group status was determined by selecting those within the upper and lower 66th percentiles for childhood maltreatment/trauma and anxiety proneness. More specifically, the possible range of scores for the CTQ (trauma questionnaire) is 0 to 96: total scores between the 0-36 range fell within the lower 33rd percentile and total scores between the range of 49 to 96, were classified as falling within the upper percentile. For anxiety proneness, the possible range of scores is 0 to 123 where a score between 0 and 78 fell in the lower 33rd bracket, and a score between 86 and 123 fell within the 66th bracket. Groups were classified as follows:

1. High childhood maltreatment/trauma + high anxiety-prone (upper 66th percentile both variables)

2. High childhood maltreatment/trauma + low anxiety-prone (Upper 66th percentile for childhood maltreatment/trauma and lower 66th percentile for anxiety proneness)

3. Low childhood maltreatment/trauma + high anxiety-prone (Lower 66th percentile for childhood maltreatment/trauma and upper 66th percentile for anxiety proneness)

4. Low childhood maltreatment/trauma + low anxiety-prone (Lower 66th percentile for both variables)

Due to a number of logistic difficulties, throughput of volunteers was initially very slow. This resulted in a number of volunteers being lost to follow-up when they either lost interest or left school. Out of the total of 1149 participants initially recruited and screened, 200 were selected based on the above criteria, of which 43 participants, in turn, participated in the current study and completed the following phases.

**3.2 Phase II:  Second level screening and assessment of potential clinical outcome modulating factors**

**3.2.1 Second level screening**

Since volunteers were required to visit the Department of Psychiatry, situated in the Tygerberg Medical School, and given their low socio-economic status, all participants were reimbursed for traveling costs, and provided with a light lunch on the day of their visit. Absentee letters to be handed in at the school were also provided. In order to control for any signs of medical illness that could confound the study results, especially in terms of the endocrine and immune parameters, a brief, general physical examination was conducted by a medical doctor. During the same visit, participants were subjected to a series of psychological assessments, as outlined in the

next few sections. For parameter choices that had not been covered extensively in the literature review, a referenced motivation and/or rationale for its inclusion is provided in the relevant section here.

### 3.2.1.1 Structure diagnostic interview

A structured diagnostic interview was conducted on the 43 participants by a qualified research psychologist (Ms Lindi Martin) to screen for the presence of anxiety and mood disorders using the Mini-International Neuropsychiatric Interview-Kid for children and adolescents (MINI-KID; (Sheehan, et al., 1998), which follows the Diagnostic and Statistical Manual of Mental Disorders-IV (American Psychiatric Association, 1994) and International Classification of Diseases-10 (World Health Organization (1992)) criteria for the diagnosis of psychiatric disorders, screening for 17 Axis I disorders. Ms Lindi Martin also conducted this interview on additional participants selected from the Phase I-narrowed-down-cohort of 200 participants, for inclusion in her study.

### 3.2.1.2 Rescreening

Phase I screening measures were re-administered to confirm group status, consisting of the following questionnaires (see Appendix B):

a. The Child Anxiety Sensitivity Index (CASI),

b. The Trait Anxiety version of the State-Trait Inventory (STAI-T), and

c. The Childhood Trauma Questionnaire (CTQ)

In addition, a neuropsychiatric assessment was conducted, also by Ms Lindi Martin, to screen for associated psychopathology by using the following self-report questionnaires:

a. The Children's Depression Inventory (Kovacs & Beck, 1997) is a 27-item self-report instrument that measures depression symptoms in children and adolescents experienced over the past two weeks. The items relate to sadness, self-blame, loss of appetite, insomnia, interpersonal relationships, and school adjustment

b. The Adolescent Drinking Inventory (Harrell & Wirtz, 1989) is a 24-item self-report measure of the severity of an adolescent's drinking problem and provides subscales that indicate the pattern of drinking-related difficulties. Drinking problem symptoms are assessed as they relate to social, psychological, and physical indicators (McPherson & Hersch, 2000)

c. Multidimensional Anxiety Scale for Children (March, et al., 1997) is a 39-item measure that taps four dimensions of childhood anxiety, namely, physical symptoms, social anxiety, harm avoidance, and separation anxiety

Data obtained from these three questionnaires were not included in the current study, as these measures served as screening measures for the presence of psychopathology, and were not used for the purpose of inclusion as potential outcome variables.

**3.2.2 Assessment of potential outcome modulating factors**

The three potential outcome modulating factors (OMFs) were measured by the following questionnaires (see Appendix C):

a. The Connor-Davidson Resilience Scale (CD-RISC) is a 25-item self-report measure that assesses the level of stress coping ability over the past month

b. The Rosenberg Self-esteem Scale (R-SES) is a 10-item self-report measure that investigates global self-worth and self-acceptance by measuring both positive and negative attitudes about the self.

c. The Edinburgh Handedness Inventory (EHI) is a 10-item scale determining handedness for assessment of cerebral lateralization.

**3.2.3 Scoring of questionnaires included in statistical analysis**

Of the above questionnaires in Phase II, the STAI trait version (STAI-T), CASI, and CTQ (section 3.2.1.2) and the CD-RISC, RSES, and EHI (section 3.2.2) were used in the performance of statistical analysis to determine the relative effects of anxiety proneness and trauma on neurophysiological outcomes as well as on the potential modulating factors (resilience, self-esteem and handedness) as well as to correlate outcomes of the STAI-T, CASI and CTQ with neurophysiological outcomes and OMFs. The process of scoring for the different questionnaires is delineated below:

a. The CASI is an 18-item self-report questionnaire with the following subscales: Physical concerns subscale: 3,6,8,9,10,11,12,14,16,18; Social concerns subscale: 1,4,5,13,17; and Psychological concerns subscale: 2,7,15. Scores on all subscales are summed for a total score.

b. The STAI-T is 40-item self-report questionnaire divided into 2 sections which assess state and trait anxiety. For group status determination, participants had only completed the 20 item 'trait anxiety' section. Positives are reversed for items 1,3,6,7,10, 13, 14, 16, 19 and scores are summed for total scores.

c. The CTQ is a 28-item retrospective measure of the frequency and severity of abuse and neglect experienced prior to age 18. The CTQ consists of 5 subscales that assess emotional, physical, and sexual abuse and emotional and physical neglect, respectively.

For the remainder of the thesis, when reference is made to trauma, it will be in the context of childhood trauma, as measured by this questionnaire. Items 2, 5, 7, 13, 19, 26, 28 are reversed scored before summation. The subscales of the CTQ consist of the following items: Emotional abuse: 3, 8, 14, 18, 25; Physical abuse:  9, 11, 12, 15, 17; Sexual abuse: 20, 21, 23, 24, 27; Emotional neglect: 5, 7, 13, 19, 28; and Physical neglect: 1, 2, 4, 6, 26. For a score of overall trauma, scores on the 5 subscales are summed.

d. For the R-SES, items 2, 5, 6, 8, 9 are reversed scored and scores are summed for a total score. The possible range of scores for this scale is 0-30. Scores between 15 and 25 are within normal range; scores below 15 suggest low self-esteem.

e. For the EHI (Fig. 3.2), preferences in the use of hands in the listed activities are indicated by putting a check in the appropriate column. Where the preference is so strong that one would never try to use the other hand, unless absolutely forced to, two checks are put in the appropriate column. In the case of indifference, one check is put in each column.  Some of the activities listed below require the use of both hands. In these cases, the part of the task, or object, for which hand preference is required, is indicated in parentheses.

The handedness score is calculated using the following formula: (Right - Left) / (Right + Left). A value of -1 denotes a pure left hander, -0.5 denotes a mixed left hander, 0 denotes a neutral hander, +0.5 denotes a mixed right hander and +1 denotes a pure right hander.

f. For the CD-RISC, scores are summed for a total score. No normal ranges are available to report here, however, scores obtained in different populations in various studies are discussed in Chapter 5, section 5.3.2.

**Figure 3.2.** The Edinburgh Handedness Inventory

|  | Left | Right |
|---|---|---|
| 1. Writing | ☐  ☐ | ☐  ☐ |
| 2. Drawing | ☐  ☐ | ☐  ☐ |
| 3. Throwing | ☐  ☐ | ☐  ☐ |
| 4. Scissors | ☐  ☐ | ☐  ☐ |
| 5. Toothbrush | ☐  ☐ | ☐  ☐ |
| 6. Knife (without fork) | ☐  ☐ | ☐  ☐ |
| 7. Spoon | ☐  ☐ | ☐  ☐ |
| 8. Broom (upper hand) | ☐  ☐ | ☐  ☐ |
| 9. Striking Match (match) | ☐  ☐ | ☐  ☐ |
| 10. Opening box (lid) | ☐  ☐ | ☐  ☐ |
| **TOTAL(count checks in both columns)** | ☐  ☐ | ☐  ☐ |
| **Difference** | **Cumulative TOTAL** | **Result** |

### 3.3 Phase III:  Basal blood draw

Phase III and IV signify the immune- and endocrine system assessments.

In order to assess basal levels of hormones and immune parameters, blood was obtained from all of the 43 participants at 08:00, at the Tygerberg Medical School. This procedure was staggered for logistical reasons, with no more than 4 participants per visit. The day's procedures commenced with participants filling out assessment forms (see Appendix D) pertaining to exercise regime, hormonal contraception, medication and illness. In the procedure room, blood was collected into four Vacutainer tubes (1x Sodium Heparin, 1x EDTA, and 2x SSTs) from each participant by a research nurse registered at the South African Nursing Council (SANC), after which the participants again received snacks, an absentee letter and a travel allowance.  At this time, an appointment for the final visit was scheduled to fall within the next 7 days, for execution of Phase IV (refer to section 3.4 for detail). The participants were also provided with medication required for Phase IV, as well as relevant instructions at this time point.

Upon arrival at the laboratory in the Department Physiological Sciences, the SST tubes were centrifuged at 1500g for 10 minutes at 4 ℃, aliquotted into reaction vials while kept on ice and frozen at -80°C until

subsequent batch analysis for circulating levels of specific hormones and cytokines as well as MPO activity. One EDTA tube per person was used for both the cell counts as well as the glucocorticoid receptor (GR) quantification.

Levels of cortisol, prolactin, dehydroepiandrosterone-sulphate (DHEAs) and testosterone were analyzed in serum, at the Pathcare laboratory, using standard techniques and laboratory practice.

Full blood counts were performed by automated analysis (Celldyne 3700 CS, Abbott).

### 3.3.1 WBC subpopulation specific GR expression

Whole blood was used for surface immunophenotyping and intracellular GR determination (See Appendix E). The staining method was modified to simultaneously label surface markers of lymphocyte subpopulations and their cytoplasmic GRs. The following panel of monoclonal antibodies (MoAb) was used: fluoresceinisothiocyanate (FITC) conjugated anti-GR; phycoerythrin (PE) conjugated anti-CD56 (staining for NK cells); Alexa Fluor anti-CD19 (staining for B cells); APC/C7 CD3 (staining for T cells); and Brilliant Violet CD14 (staining for monocytes) (BiocomBiotech).

### 3.3.1.1 Instrument setup

The BD FACSAria flow cytometer (BD Biosciences, USA) was employed for the analysis of samples. This instrument is a high-speed fixed-alignment benchtop cell sorter that can be operated at varied pressures to acquire up to 70,000 events per second. It enables multicolour analysis of up to 13 fluorescent markers and two scatter parameters, forward scatter (FSC) and side scatter (SSC). It is equipped with blue (488-nm), red (633-nm) and violet (405-nm) solid state lasers for excitation of fluorochrome-conjugated antibodies. It is also equipped with two types of signal detectors; photomultiplier tubes (PMTs) and a photodiode detector. PMTs detect signals generated by SSC and all fluorescence channels whereas the photodiode detects signal generated by FSC.

The BD FACSAria II possesses both octagon and trigon detector arrays. The octagon detector array contains six PMTs that detect SSC and up to seven fluorescence signals excited by the blue laser. The trigon arrays detect fluorescence signals excited by the red and violet lasers, respectively. Each trigon contains two PMTs that detect up to three fluorescence channels. The PMTs within each array convert light into electrical pulses that can be

processed by the electronics system and converted into data. Acquisition and data analysis are performed and controlled by BD FACSDiva software (BD FACSAria User Guide).

Fluorescence spillover was analysed by use of fluorescence minus one (FMO) controls for each monoclonal antibody conjugate included in the 5–colour staining panel. FMO controls were employed in order to determine the demarcation of the true positive and negative populations for the markers of interest. For the compensation, Anti-Mouse Ig CompBeads were employed (BD Biosciences, USA). Single-stained bead controls were prepared for each antibody in the staining panel and stored overnight at 4˚C, protected from light. Automated compensation was performed on the samples, using the algorhythm provided in the BD FACSDiva v6.1.3 software.

Unfortunately, due to either poor sample quality (delay in getting the sample to the lab) or technical error, the samples in this study showed more inter-individual variation than expected in signal intensity for CD45 specifically. Thus, we thought it more responsible to not set a threshold on the CD45 gating plot and to rather gate and acquire all cells, consider the whole plot and analyse samples manually after the complete dataset had been acquired. In our opinion, setting a threshold in this case may have led to inappropriate exclusion of some cells. After manual gating for the CD45+ population, the CD19 and CD14 cells were gated for within this CD45 population. The CD19-/CD14- population was then the parent gate to identify CD3+ and CD56+ cells. This stepwise gating procedure was employed, since it yielded the best possible population separation for our samples.

Absolute cell counts were calculated for leukocyte subpopulations that were analysed using flow cytometry. These were derived from the total WBC and lymphocyte count obtained from the automated hematology analyzer (Celldyne), as well as the respective percentages of cells of the lymphocyte gates obtained during flow cytometry analysis.  Furthermore, as a quality control (QC) step, the calculated absolute counts of subpopulations were also added and did sum to the total lymphocyte count (as obtained from hematology data).

At this point, it is also necessary to point out a limitation of the flow cytometry technique used for GR Mean Fluorescent Intensity (MFI) assessment: currently, the use of Molecules of Equivalent Soluble Fluorochrome (MESF) beads for standardisation is required for this type of analysis. However, at the time of protocol design (2010) and initiation of actual sample collection (2011), the MESF bead standardisation was not common practice yet – to our knowledge, these guidelines were only published in 2013 (Tanqri, et al., 2013). As it was not possible to change the protocol halfway through, especially given the already low sample number, the

original protocol was maintained. We acknowledge that this may have resulted in somewhat compromised data, but opted to not exclude it, but to still present it for the purpose of the thesis, to illustrate the complexity of the study design and integrated interpretation.

### 3.3.2. Pro-inflammatory marker assessment

Frozen serum was thawed on ice and MPO activity assessed *via* an enzyme linked immune sorbent assay (ELISA) (abcam AB119605 Myeloperoxidase (MPO) Human ELISA, Biocom Biotech). The detection limit of this assay was 0.312 ng/ml.

In order to determine dominance of an either Th1 or Th2 phenotype associated with anxiety proneness and or/trauma exposure, we measured levels of IL-12p70 as well as levels of IL-8, IL-1β, IL-6, IL-10, and TNF-α in serum using a kit (BD/551811, Becton Dickinson (BD) Cytometric bead array (CBA) Human Inflammatory Cytokines Kit). The respective detection limits were 1.9 pg/ml for IL-12p70, 3.6 pg/ml for IL-8, 7.2 pg/ml for IL-1β, 2.5 pg/ml for IL-6, 3.3 pg/ml for IL-10 and 3.7 pg/ml for TNF-α.

### 3.4 Phase IV: Betamethasone suppression test with subsequent psychosocial stress test

To our knowledge, only one other study (Carpenter, et al., 2007) has assessed TSST reactivity in association with Dex/betamethasone suppression. Preparation for the TSST for groups involved setting up 8 screens with chairs in between, in our conference room at the Department of Psychiatry, and organizing with an in-house researcher and psychiatrist on the same floor to act as the two confederates.  A trial run of the TSST was also performed on 4 student volunteers to ensure that everything would run smoothly on the actual test day.

Briefly, each participant received two 0.5mg tablets of Betamethasone (equivalent to Dexamethasone, which is no longer available in South Africa) and were instructed to not exercise the day before the phase IV visit, to take the tablets that evening at 11h00pm, and to arrive at the department the next day in a fasted state, including having had to refrain from drinking coffee from the time they had woken up, as well as for the entire duration of the TSST. Upon arrival, a maximum of 7 participants filled in the same assessment form as the one administered at the basal blood draw visit, in addition to the state version of the STAI and the Visual Analogue Scale (VAS) for anxiety (see Appendix F). Blood (one EDTA and one SST Vacutainer tube per participant) was drawn in the procedure room and kept on ice until subsequent processing, which was done within 2 hours from venipuncture. Once all the forms had been filled in and all participants' blood drawn, the instructions for the stress test were read to the participants in the waiting room. The participants were also instructed to go to the procedure room

immediately after the stress test, for another blood draw, one participant at a time. (All blood samples were centrifuged at 1500g for 10 minutes at 4 ℃, after which serum and plasma were aliquotted into reaction vials and stored at -80°C for batch analysis of cortisol and ACTH levels by Pathcare.) The participants were given 10 minutes to prepare for their 2-minute speeches. Then, the participants were escorted to the conference (testing) room and instructed to take a seat. Participants were called out in a random fashion by one of the confederates. Each participant was instructed to first deliver his/her speech, after which he/she completed the arithmetic task. (For the TSST procedure, instructions and debriefing, see Appendix G.)

At the end of the TSST, subjects were debriefed and asked to do the post-TSST VAS by the research nurse. No participant reported any adverse effects during any of the Phase IV procedures.  Participants were given snacks and lunch from the cafeteria, their travel allowances and absentee letters before departure. 42 out of the 43 participants from whom a basal blood draw was collected also completed Phase IV.

Scoring of the STAI-S involved that the scores for the 20 items of the STAI that were associated with state anxiety were added to obtain a total score. (This was done after correcting scores that had been reversed for anxiety-absent items, namely items 3, 4, 6, 7, 9, 12, 13, 14, 17, 18.) The range of scores for this instrument is usually between 20–80, with the higher score indicative of greater anxiety. A cut-off point of 39–40 has been suggested to detect clinically significant symptoms for the State Anxiety scale, while other studies have suggested a higher upper threshold score of 54–55 for older adults.

The VAS is a continuous measurement of subjective current anxiety on a scale from 0 to 10, where higher scores indicate more anxiety. The VAS is of most value when looking at change over time, within individuals, rather than comparing scores across a group of individuals at one time point.

### 3.5 Phase V: Neurocognitive measures and Magnetic Resonance Imaging (MRI)

### 3.5.1 Neurocognitive measures

Of the 43 participants from whom we collected basal blood samples, 39 underwent neurocognitive testing using a comprehensive test battery. Due to time constraints, we were able to obtain preliminary data from 33 of the 39 participants that have undergone psychometric assessments, to include as results in Chapter 4. The current study was run in parallel to the other thesis study already mentioned, which made use of a comprehensive array of neurocognitive measures. However, elaborate neurocognitive testing was beyond the scope of the current study and we therefore chose to include data from only 2 measures of the Senior South African Individual Scale

(revised) test (SSAIS), one measure form the Wechsler memory scale (Revised) (WMS-R) and all the measures of the Tower of London (TOL).  For statistical analysis, we used raw scores as opposed to norm scores, since the latter may not be representative of specifically our study's South African socio-demographic population.

For the SSAIS, we used the SSAIS Digits Backward Total as an indication of verbal working memory and left-hemisphere function, where higher scores indicate better performance. Here, 'verbal' refers to the fact that with this test, a series of digits are presented to the participant in an auditory fashion after which they have to repeat the digits verbatim, in reversed sequence. This measure tests auditory short term (30 seconds) memory for numbers, as well as more "disguised" functions of mechanical memory such as attention and concentration, as these functions are required to perform this test. The SSAIS Block Designs Total score was used to broadly indicate executive (PFC) function and here again, higher scores indicated better performance. The block design test consists of 15 items where 4 plastic cubes were used by the participant to copy increasingly difficult patterns from the first seven items, and nine blocks were used for the remaining. More specific cognitive functions employed during the performance of this test include: non-verbal intelligence, non-verbal problem solving skills, perceptional organization, spatial visualization and orientation, concentration, visual-motor co-ordination and abstract conceptualization.

For the WMS-R, an Immediate Recall Overall Total (WMS-IR overall total) score has been used to indicate visuospatial working memory as a function of the right hemisphere. With this test, the participant was presented with an image which he/she was required to reproduce from memory immediately after presentation.

Performance on the Tower of London is potentially associated by neurosubstrates allocated to the mid-dorsolateral frontal cortex and the ventrolateral cortex (Owen, 1997). The test consists of two boards with three pegs and three different colored beads each. With this test, the examiner presents a specific format of the beads to be reproduced by the participant in as few moves as possible. Data was obtained for the following variables:

a) TOL total correct - higher scores indicate better performance. This score indicates the number of times the participant has managed to complete a trial in as few moves as is allowed. For example, if the lowest possible number of moves is '4' and he/she completes it in 4 moves.

b) TOL total move score – lower scores indicate better performance. A move is recorded when a bead is completely moved from a peg and placed on either the same peg or another peg. The primary TOL score is

based on the total move score which is the sum of the move scores for each of the individual test problems and ranges from 0 to a maximum of 149 for the Child Recording form.

c) TOL total initiation time - lower scores indicate better performance. This score indicates the time from when the participant is presented with the trial and his/her first move to start the trial and assesses inhibitory response processes ranging from under-to over-controlled. In contrast to the other time score, a high total initiation time score correspond to slower initiation times and more mature and thoughtful preparation to a problem.

d) TOL total execution time - lower scores indicate better performance. This score indicates the ability to complete the task in a certain time and measures the speed at which executive plans are operationalized. This score gives information pertaining to the level of executive planning and problem solving upon strategy implementation.

e) TOL total time - lower scores indicate better performance. This score gives an indication of overall executive planning in relation to overall problem solving speed.

**3.5.2 Functional and structural MRI scan**

Of the 43 participants who have provided blood for the basal blood draw, 34 participants completed a structural and functional MRI. Due to time constraints, we present only data for the structural MRI, which were processed in time.

**3.5.2.1 Functional MRI**

On the day of the scan, participants were required to practice the neurocognitive test to be used in the scanner, about 2 hours in advance of the scan. This practice run was performed by doing a trial run of the International Affective Processing Scale task (IAPS) on a computer, with the participants using the keys on the keyboard in their responses, which corresponded to the buttons used in response box in the scanner. The IAPS entailed the following:

The IAPS task allows one to assess brain activation, with particular focus on the amygdala and hippocampus, during basic emotion processing. The task consists of the presentation of images/pictures from the IAPS which is categorized into three conditions, i.e. positive, neutral and negative, according to validated ratings of the IAPS. The task contains four activation blocks consisting of 96 seconds each, interleaved with four baseline rest blocks (i.e. a fixation cross on the screen for 32 seconds). Eight pictures/images of each condition (positive,

neutral and negative) are presented in pseudo-random order. Therefore, each of the four activation blocks contains 24 images/pictures and participants were instructed to view each picture for two seconds and rate it (for a maximum time of 2 seconds). The duration of the task in the scanner was 9 minutes. Stimuli as part of the task administered were computerized and displayed to the participant in the scanner *via* a screen display. The protocol used sequential task activation in which stimuli alternate between active and resting states. This enabled a number of approaches to statistical analysis of the functional data. Furthermore, generally, the repeated acquisition of data for each active task segment improves power. More specifically, it improves the ability to detect what may amount to relatively small changes in signal.

Other imaging procedures included the Diffusion Tensor Imaging (DTI) and a resting state period. The DTI measures the random translational motion of molecules that result from the thermal energy carried by these molecules. DTI, an MRI variation, allows for diffusion anisotropy effects in diffusion MRI data to be extracted, characterized and exploited, providing details on tissue microstructure (in particular, white matter tract area) and connectivity of tissue structures, thereby improving understanding of brain structure and neural connectivity. In addition to the DTI, a resting state period was also included. Imaging the brain during rest reveals large-amplitude spontaneous low-frequency (<0.1Hz) fluctuations in the fMRI signal that are temporally correlated across functionally-related areas. These correlations, referred to as 'functional connectivity' yield thorough maps of complex neural systems, collectively constituting an individual's 'functional connectome'. A brief (approximately 5 minutes), single resting state fMRI scan was used to assess a number of functional circuits concurrently.

Data were acquired on a MAGNETOM Siemens Allegra syngo MR 3 Tesla scanner, with a 4 channel head coil, at the Cape Universities Brain Imaging Centre (CUBIC), at the Faculty of Health Sciences, Tygerberg. A high resolution T1-weighted 3D MPRAGE structural image was obtained with the following parameters: sagittal orientation, TR =2300ms, TE = 3.93ms, FOV = 220mm, 160 slices; 256x256 matrix; 1x1x1mm^3 resolution; 9 minute scan time. Echo planar imaging was used for the fMRI and acquired in a tilted axial orientation with TR = 2000ms, TE = 30ms, 200mm field of view, 64x64 matrix, and 34 slices. Data was analyzed using FSL (FMRIB, Oxford, UK). There were 2 imaging epochs (one for each cognitive affective paradigm).

Images were corrected for movement, and general linear trends were removed. Correlating time-series signal data from each voxel with a reference waveform that corresponds to the timing of the test conditions allowed voxelwise percentage change in signal intensity to be calculated by dividing the average difference between

signal during the activation period and the normalized baseline by the total baseline (resting) average to derive a functional activation map for each individual. This map was overlaid onto each participant's anatomical image.

Unfortunately, due to the complexity of data analysis, these data could not be included in this thesis and will not be presented in the Results section.

### 3.5.2.2 Structural MRI

The following imaging parameters were used: TR = 2530ms; TE = 1.53ms; TI = 1100ms; flip angle = 7 degrees; voxel size = 1.3x1.0x1.3mm; field of view = 256mm. The brain imaging software package, Freesurfer, was used to acquire region specific grey matter volume measurements in different brain regions for comparison purposes between the left and right hemispheres in the amygdala, hippocampus, thalamus, and PFC. In general, Freesurfer is used for the study of cortical and subcortical regions (Dale, et al., 1999; Fischl & Dale, 2000) and a great benefit of this software is that it is fully automated. However, it is computationally intensive. Therefore, the Centre for high performance computing (CHPC) in Rosebank, Cape Town and its custom batching scripts were used to streamline this process.

### 3.6 Statistical analysis

Two-way ANOVA was used to investigate the effect of trauma and anxiety exposure on the neuroendocrine and OMF measures. For post hoc testing, Fisher least significant difference (LSD) tests were used. For univariate comparisons of the measurements, Spearman correlations were used. A 5% significance level ($p<0.05$) was used as guideline for determining significance.

Mixed model repeated measures ANOVA was used to compare the effects of trauma and anxiety on measurements obtained pre- and post-TSST. In these analyses gender, anxiety, trauma and time was used as fixed effects, with subject nested in gender*trauma*anxiety as random effect.

# CHAPTER 4

# RESULTS

We have structured the presentation of results in four main components – the first three components are of descriptive cross-sectional data, while the fourth component represents an intervention (Betamethasone suppression and challenge). Firstly, we present the data used to psychometrically profile all study participants in terms of trauma and anxiety proneness – the measures used with which to group participants for group comparisons. Secondly, we present physiological (endocrine and immune) and neurocognitive parameters as group comparisons. Thirdly, the participant cohort as a whole was used in statistical analysis to determine relationships between specific parameters over the spectrum. Lastly, we present data on the intervention. Results are presented as means and standard errors of the mean, unless otherwise specified.

## 4.1 Psychometrical profiling

### 4.1.1 Profile in terms of anxiety proneness and trauma exposure

The validity of questionnaires used for grouping of individuals into the four experimental groups in the population studied – according to trait anxiety (STAI-T), anxiety sensitivity (CASI) and trauma exposure (CTQ) – was evident from results obtained. While the STAI-T score was dependent on both anxiety proneness (ANOVA main effect, $P < 0.000001$) and trauma exposure (ANOVA main effect, $P < 0.01$), the CASI total score and CTQ total score exhibited specific dependency for only anxiety proneness (ANOVA main effect, $P < 0.00001$) and trauma (ANOVA main effect, $P < 0.00001$) respectively.  Identical dependencies were recorded for all sub-scores in both questionnaires. These are summarized in Table 4.1.

**Table 4.1.** Effect of trauma exposure and anxiety proneness on trait anxiety, anxiety sensitivity and -subscales and trauma and –subscales

|  | ANOVA effect of trauma exposure | ANOVA effect of anxiety proneness |
|---|---|---|
| **STAI-T** | **↑, p < 0.01** | **↑, p < 0.000001** |
| **CASI total** | -, p = 0.17 | **↑, p < 0.000001** |
| CASI Social Concerns | -, p = 0.87 | **↑, p < 0.001** |
| CASI Psychological   Concerns | -, p = 0.93 | **↑, p < 0.0001** |
| CASI Physical Concerns | **↓, p = 0.05** | **↑, p < 0.000001** |
| **CTQ total** | **↑, p < 0.00001** | -, p = 0.14 |
| CTQ Physical neglect | **↑, p = 0.01** | -, p = 0.27 |
| CTQ Emotional abuse | **↑, p < 0.001** | -, p = 0.20 |
| CTQ Emotional neglect | **↑, p < 0.01** | -, p = 0.72 |
| CTQ Physical abuse | **↑, p < 0.001** | -, p = 0.23 |
| CTQ Sexual abuse | **↑, p = 0.03** | -, p =1.00 |

Significant p-values are indicated in bold. Arrows indicate the direction of the significant relationships.

### 4.1.2 Potential modulators: handedness, self-esteem, resilience

Parameters selected as potential outcome modulators of trauma and/or anxiety induced endocrine and immune adaptations were analysed in context of anxiety proneness and trauma.

A brief revision of the scoring for handedness is provided: Degree of hand preference (consistent *versus* inconsistent), was determined using the Edinburgh Handedness Inventory (EHI). A score of zero indicates the highest degree of inconsistent (mixed) handedness, negative scores from 0 to -1 would indicate increasingly consistent left-handedness, while increasingly consistent right-handedness would range on the positive axis from 0 to 1. All individuals assessed in the current study proved to be right-handed, although across a wide range (minimum 0.2 to maximum of 1). Neither anxiety, nor trauma had any effect on this measure.

For self-esteem, the Rosenberg Self-esteem Scale (R-SES) was employed. A score of 15-25 is deemed normal, while a score of less than 15 would indicate low self-esteem. In the 43 individuals assessed in the current study, all had scores within the normal range. When considering differences between groups, trauma oddly seemed to increase self-esteem in this population (ANOVA main effect of trauma, P<0.05), however, this was only true for high-anxiety individuals (low trauma exposure and high anxiety proneness (LO-HI): $19.6 \pm 3.2$ vs. HI-HI: $23.8 \pm 3.6$, P<0.05), while low-anxiety individuals did not show an effect of trauma (LO-LO: $20.3 \pm 5.7$ vs. HI-LO: $22.3 \pm 3.9$, P=0.29).

In general, resilience in the past month, as assessed by the Connor-Davidson Resilience Scale (CD-RISC), seems to be population specific in terms of "normal range" (discussed in more detail in the Chapter 5), so that it is mostly interpreted in comparison to a control group, which, in our study would be the LO-LO group. (A higher score would generally indicate a higher level of resilience.) Although neither trauma, nor anxiety proneness showed significant effects on this measure (which also showed relatively high variability), there was a tendency for trauma exposure to decrease resilience (ANOVA main effect, P=0.07 and post hoc testing, LO-LO: $73.4 \pm 13.8$ vs. HI-HI: $58.6 \pm 14.6$, P=0.08).

### 4.2 Basal blood analysis

### 4.2.1 Endocrine profile

Prolactin (normal range: 2.6 - 13.1 µg/L) cortisol (normal range for morning samples: 184-618 nmol/L) and DHEAs (normal range: 4.34 -12.2 µmol/L) levels were not influenced by gender, so the results are presented for

the group as a whole for these parameters (Fig. 4.1 a, b, and c). While serum prolactin levels were not influenced by either trauma or anxiety proneness, effects of especially anxiety proneness were observed for both cortisol and DHEAs. Anxiety proneness showed a tendency to reduce basal serum cortisol, independently of trauma (ANOVA main effect, P=0.07), as well as a significant effect to decrease basal serum DHEAs levels (ANOVA main effect, P<0.05). The latter effect was largely due to a major inhibitory effect in anxiety prone individuals without trauma (Fischer LSD post hoc LO-LO vs LO-HI, P=0.01). Of further importance is the fact that a large portion of this population presented with below-normal DHEAs concentrations: 67% of HI-HI, 75% of HI-LO, 100% of LO-HI and 62% of LO-LO. Except for one individual with a slight hypocortisolaemia (LO-HI, 120 nmol/l) and 5 with slightly elevated prolactin levels which were distributed across groups, all other hormone concentrations were within the normal reference limits as provided by Pathcare South Africa.

**Figure 4.1.** Effect of anxiety proneness on serum (a) prolactin, (b) cortisol and (c) dehydroepiandrosterone sulfate (DHEAs) levels (Error bars denote SEM)
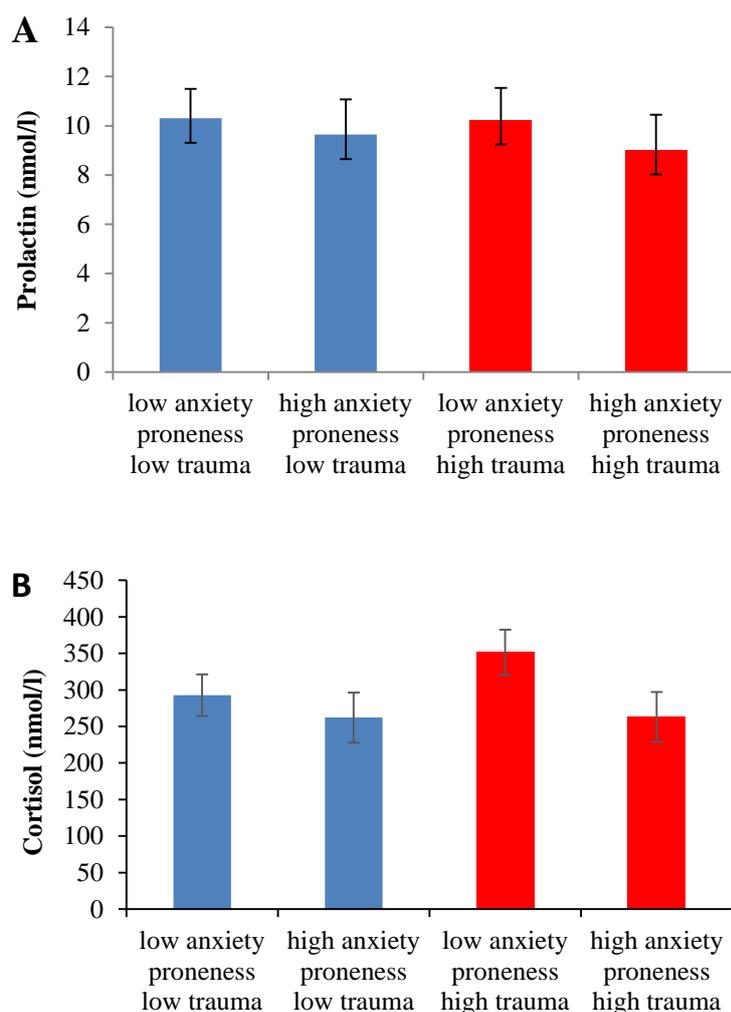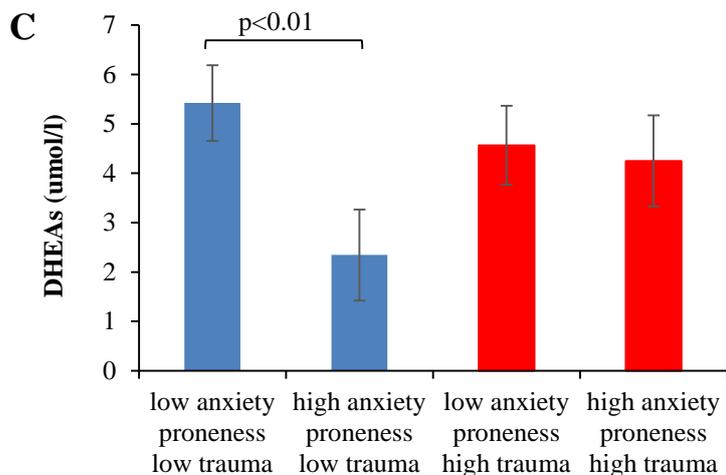
For testosterone (normal range: 9.9 - 27.8 nmol/L) only, there was a main effect of gender, as anticipated, with males exhibiting significantly higher basal testosterone levels than females ($21.8 \pm 1.3$ vs. $2.3 \pm 1.0$ nmol/L, ANOVA main effect $P < 0.00001$). Testosterone was not further analysed statistically for males, since the number of participants of this gender was in our opinion insufficient for group comparison (n=11). In the female group, testosterone levels did not show relationships with either trauma or anxiety proneness. However, since testosterone levels are normally relatively low in females, we cannot draw firm conclusions in the absence of male data.

**4.2.2 Full and differential leukocyte counts**

Leukocyte counts were all within normal reference ranges (Table 4.2).

**Table 4.2.** Descriptive statistics for WBC counts

| | Average cell count (x10$^9$/L) | SD | Normative reference ranges (count x10$^9$/L) |
|---|---|---|---|
| Total WBCs | 5.88 | 1.47 | 4.0 - 11.0 |
| Neutrophils | 3.38 | 1.38 | 2.5 - 7.5 |
| Lymphocytes | 2.00 | 0.58 | 1.5 - 3.5 |
| Monocytes | 0.29 | 0.18 | 0.2 - 0.8 |
| Eosinophils | 0.17 | 0.17 | 0.04 - 0.4 |
| Basophils | 0.03 | 0.02 | 0.01 - 0.1 |

Total white cell counts as well as neutrophil counts were similar across all groups and did not seem to be affected by either trauma or anxiety proneness.  In contrast, the total lymphocyte count was significantly decreased by anxiety proneness (ANOVA main effect, P=0.01) but not trauma, although there was a significant interaction effect (P=0.001, Fig. 4.2a), which suggested that in low-trauma, this effect was not as evident. This was confirmed by Fischer post hoc tests, which showed a significant difference only between HI-LO and HI-HI ($2.41 \pm 0.55$ vs. $1.50 \pm 0.52$ x$10^9$/l, P<0.001). When we considered lymphocyte subpopulation distribution in these groups, more specific information came to light. B lymphocytes and NK cells were not affected by either trauma or anxiety proneness.

In terms of T cells, it is necessary to explain our analysis of flow cytometry data first. On analysis, a double-population of CD3 positive cells was observed on the flow cytometry scatter plots for all subjects. On consultation with the literature (Katchar, et al., 2005; Lanier, et al., 1986; Schmidt, et al., 1986), it became evident that this double population was as a result of the incomplete separation of the CD3+ subpopulation of cells known as NKT cells – a subpopulation which in itself is quite heterogenous, as reviewed in section 2.6.5. Both NKT and T cell populations are CD3 positive, but in addition, NKT cells are also CD56 positive. In retrospect, the specific CD56 antibody used may not have been optimal for our purposes, since it failed to separate the CD56 positive NKT from the CD 56 negative T cells. Other clones of CD56 antibodies, or additional markers specific to relevant NKT subpopulations, will be considered in future to resolve this issue. However, sample unavailability excluded the possibility of repeating this analysis on current study samples. Thus, since there was no way of accurately distinguishing the NKT population from the T cell population, and since isolation of the NKT population was not vital for the purpose of this thesis, these two populations were grouped together under the heading "T cells". For the purpose of the thesis, re-analysis of all samples was unfortunately not possible, although this will be completed for publication purposes. Therefore, we are unable to provide T-cell GR data, but corrected T-cell counts and applicable statistical results are presented below.

Returning to our results, total T lymphocyte count showed the most significant effect of anxiety proneness to decrease absolute cell count (ANOVA main effect, P=0.01). This effect was most pronounced in the high trauma group, where significant differences in cell counts were observed between low and high anxiety proneness groups (Fig. 4.2b), although statistically, no main effect of or interaction with trauma was evident.

**Figure 4.2.** Effect of anxiety proneness and trauma on (a) total lymphocyte count and (b) total T lymphocyte count (units are all cells x$10^9$/L) (Error bars denote SEM)
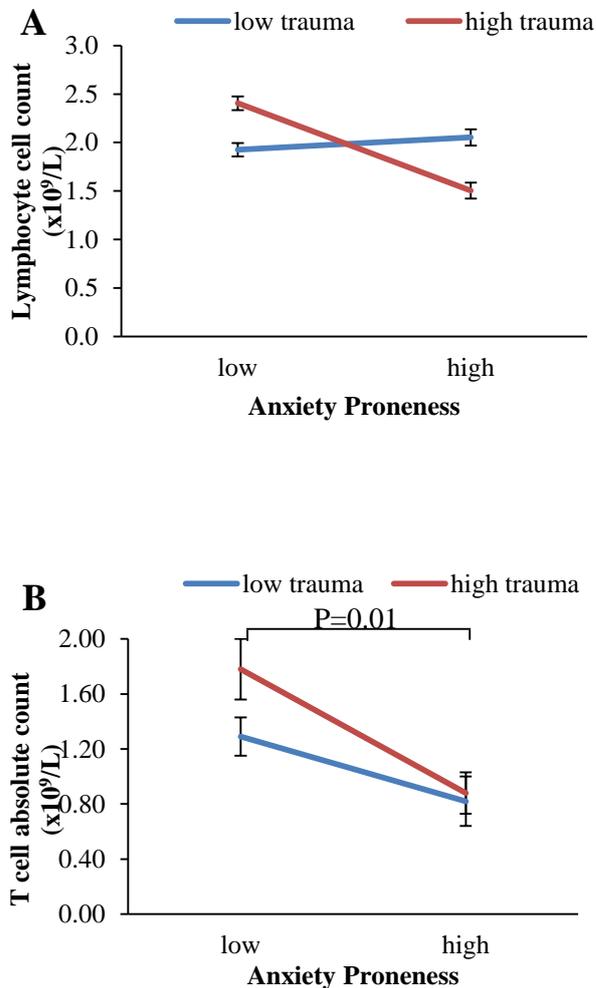


Turning attention to monocytes, again anxiety proneness was associated with a significantly lower monocyte count (ANOVA main effect, $P<0.001$), and again with no evident effect of trauma (Fig. 4.3). While the basophil count was similarly affected by anxiety proneness (ANOVA main effect, $P<0.001$), in addition, this cell type also showed a tendency for trauma to increase counts ($P=0.06$). Despite this tendency, for both low and high trauma conditions, post hoc tests indicated significantly decreased basophils counts in association with anxiety proneness (HI-HI vs. HI-LO and LO-HI vs. LO-LO both $P<0.01$)(Fig. 4.4). Eosinophils were not affected by either trauma or anxiety proneness.

**Figure 4.3.** Effect of anxiety proneness to decrease monocyte count (Error bars denote SEM)
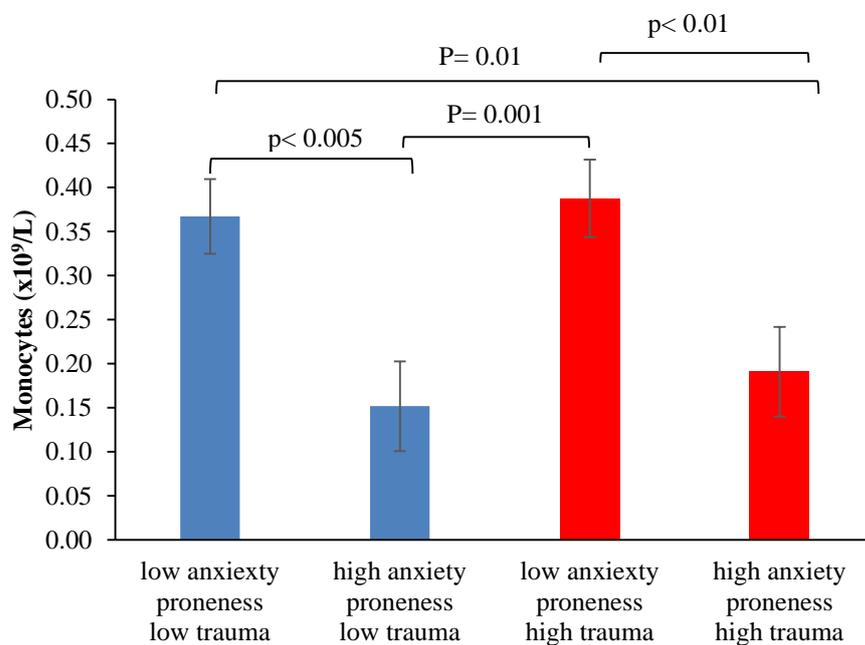


**Figure 4.4.** Effect of anxiety proneness to decrease basophil count (Error bars denote SEM)
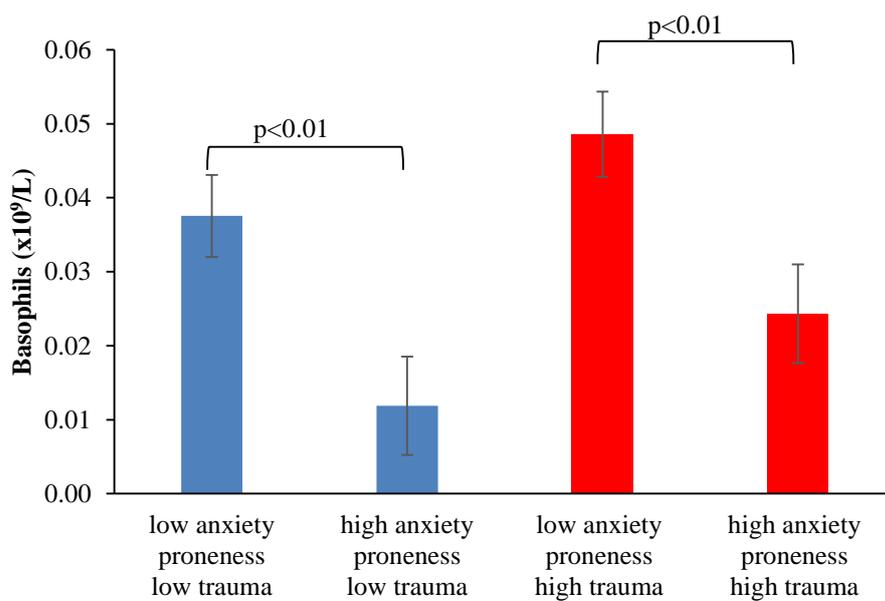
### 4.2.3 Leukocyte glucocorticoid receptor expression

Given the differential effects of trauma and anxiety proneness on cell count reported for the different subpopulations of lymphocytes, GR expression was assessed in these subpopulations individually (Table 4.3). A representative flow cytometry analysis is provided in Figure 4.5.

**Table 4.3.** Effect of anxiety proneness on GR expression (expressed as mean fluorescent intensity) in selected subsets of WBCs

|  | ANOVA effect of trauma exposure | ANOVA effect of anxiety proneness |
|---|---|---|
| B lymphocyte GR | $p = 0.42$ | **↑, p < 0.05** |
| NK cell GR | $p = 0.52$ | **↑, p = 0.01** |
| Monocyte GR | $p = 0.43$ | **↑, p = 0.005** |

Significant p values are indicated in bold. Arrows indicate the direction of the significant relationships.

**Figure 4.5** Representative flow cytometry result to illustrate gating, (a) showing original gating and (b) T cells including NKTs



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**FACSDiva Version 6.1.3**



30 October 2014-MM-19-Wall Stained
B-lymphocytes GR+
GR FITC-A

30 October 2014-MM-19-Wall Stained
Monocytes GR+
GR FITC-A

30 October 2014-MM-19-Wall Stained
NK cells GR+
GR FITC-A

30 October 2014-MM-19-Wall Stained
T-Lymphocytes GR+
GR FITC-A

30 October 2014-MM-19-Wall Stained
NKT cells GR+
GR FITC-A

30 October 2014-MM-19-Wall Stained
CD56 PE-A
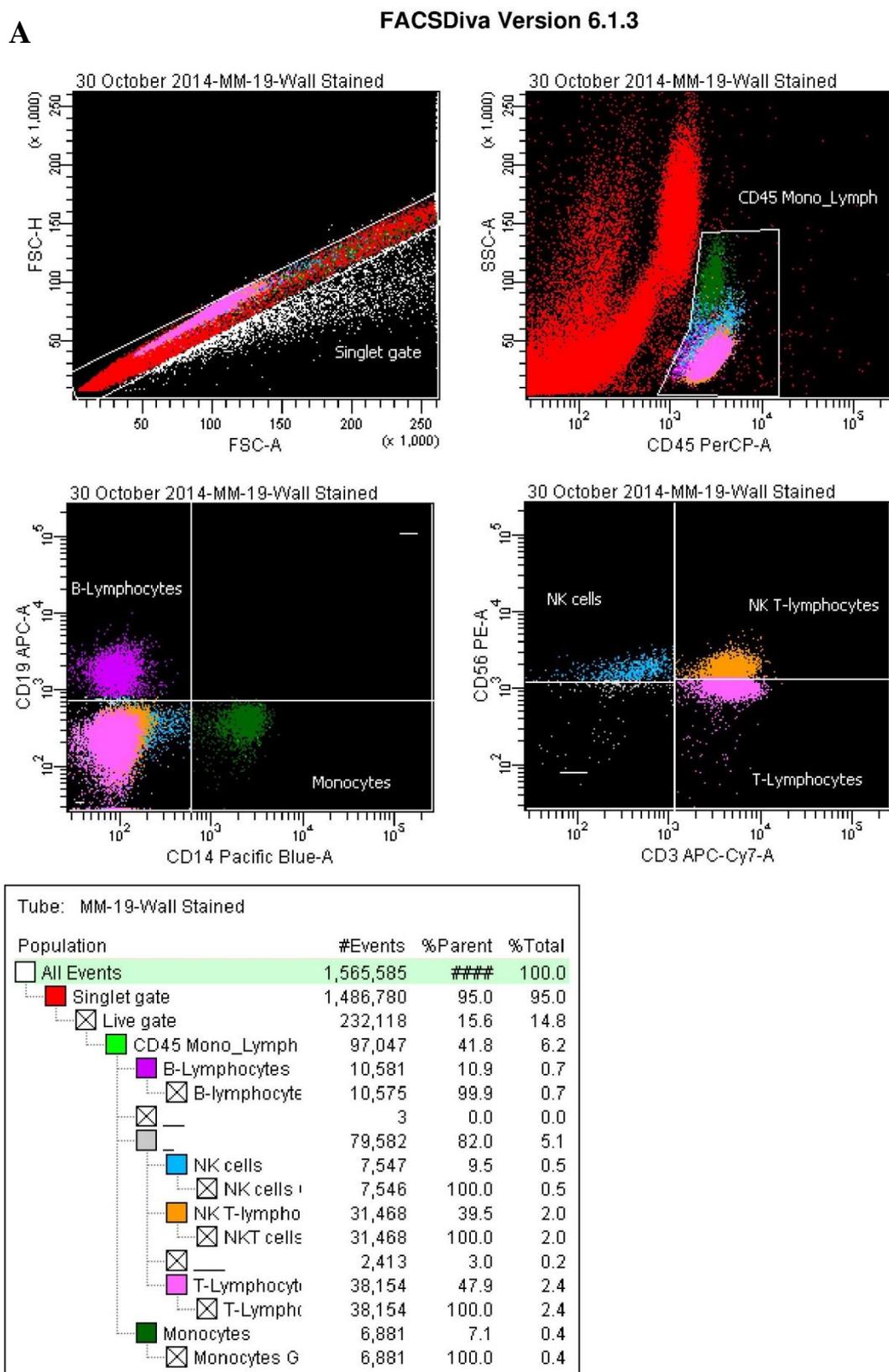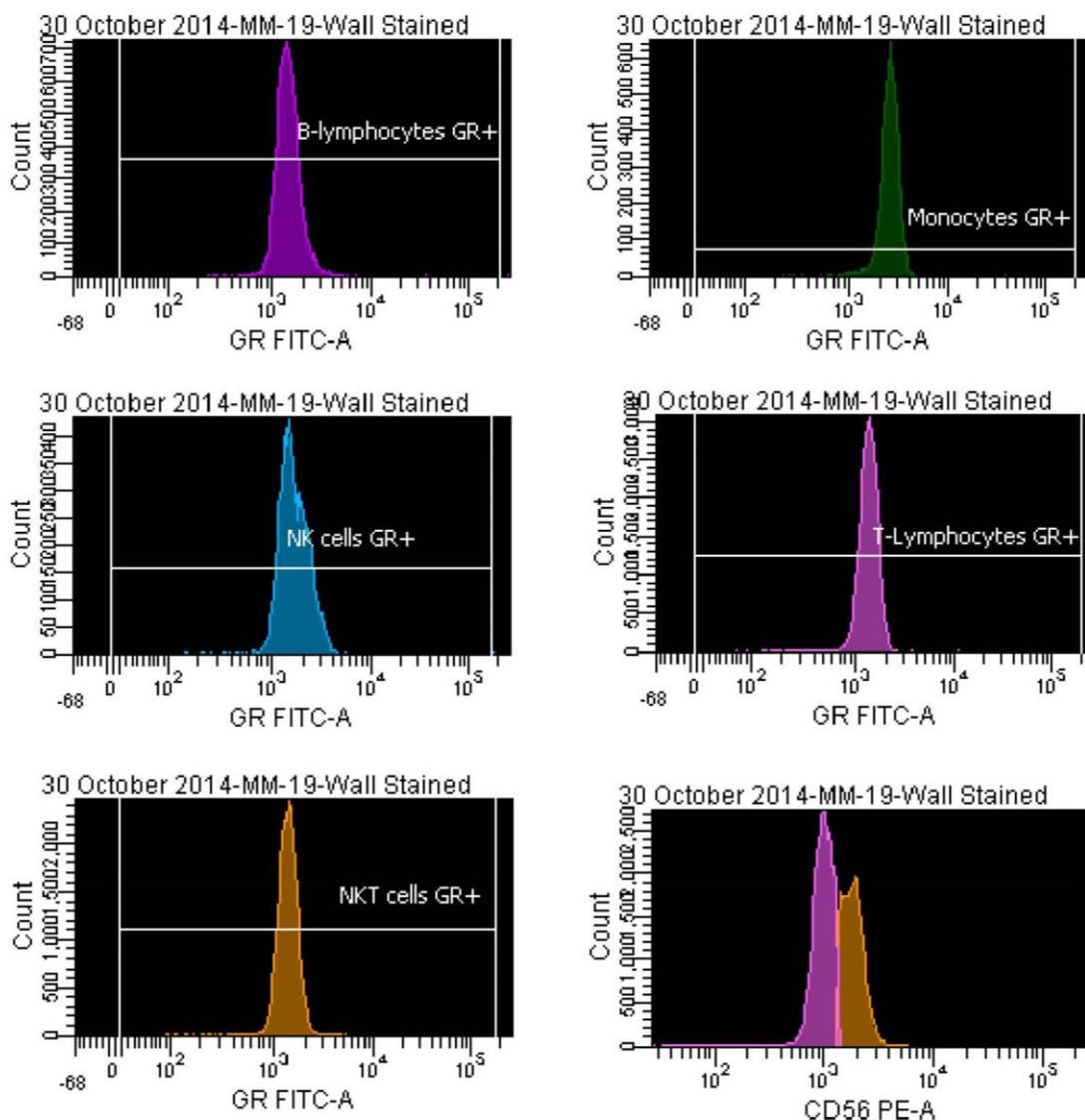
Experiment Name:  Patient samples 22 October 2014
Specimen Name:    30 October 2014
Tube Name:        MM-19-Wall Stained

| Population | GR FITC-A Geo Mean | GR FITC-A Median |
|---|---|---|
| B-Lymphocytes | 1,406 | 1,380 |
| B-lymphocytes GR+ | 1,402 | 1,380 |
| NK cells | 1,613 | 1,545 |
| NK cells GR+ | 1,612 | 1,544 |
| NK T-lymphocytes | 1,325 | 1,328 |
| NKT cells GR+ | 1,325 | 1,328 |
| T-Lymphocytes | 1,311 | 1,323 |
| T-Lymphocytes GR+ | 1,311 | 1,323 |
| Monocytes | 2,453 | 2,506 |
| Monocytes GR+ | 2,453 | 2,506 |

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**B**

**FACSDiva Version 6.1.3**



| Tube: MM-19-Wall Stained | | | |
|---|---|---|---|
| Population | #Events | %Parent | %Total |
| All Events | 1,565,585 | #### | 100.0 |
| Singlet gate | 1,486,780 | 95.0 | 95.0 |
| Live gate | 232,118 | 15.6 | 14.8 |
| CD45 Mono_Lymph | 97,047 | 41.8 | 6.2 |
| B-Lymphocytes | 10,581 | 10.9 | 0.7 |
| B-lymphocytes GR+ | 10,575 | 99.9 | 0.7 |
| __ | 3 | 0.0 | 0.0 |
| _ | 79,582 | 82.0 | 5.1 |
| - | 9,946 | 12.5 | 0.6 |
| T-lymphocytes | 69,610 | 87.5 | 4.4 |
| T-Lymphocytes GR+ | 69,610 | 100.0 | 4.4 |
| -- | 14 | 0.0 | 0.0 |
| - - | 12 | 0.0 | 0.0 |
| NOT(T-lymphocytes) | 9,972 | 12.5 | 0.6 |
| --- | 10 | 0.1 | 0.0 |
| ---- | 2 | 0.0 | 0.0 |
| ----- | 838 | 8.4 | 0.1 |
| NK cells | 9,122 | 91.5 | 0.6 |
| NK cells GR+ | 9,122 | 100.0 | 0.6 |
| Monocytes | 6,881 | 7.1 | 0.4 |

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**FACSDiva Version 6.1.3**



Anxiety proneness was associated with an increased expression of GR in all subpopulations assessed (ANOVA main effect of anxiety, all P<0.05). Trauma did not influence GR expression statistically. However, it did seem to have an effect on the magnitude of increase associated with anxiety. In the two groups with low anxiety proneness, values for GR expression were similar, i.e. independent of trauma. However, in the presence of higher anxiety proneness, the anxiety-associated difference in GR expression was on average ≈2-3-fold higher in the low-trauma vs. the high-trauma group (Fig. 4.6a-c), suggesting that trauma exposure may blunt the GR response to anxiety to some extent.

Additionally, from these figures it is interesting to note that in the LO-LO group, GR expression is similar in all cell populations reported on here. (Although the graphs for T cells are not shown below, both the original "NKT" and "T" populations showed similar results to those of the other leukocyte populations, so that the assumption can be made that the complete T cell response will correspond to that shown for the other leukocytes below.) However, when assessing the difference in counts (all increased) associated with anxiety, B cells

showed the smallest relative "GR response" at ≈20-50%, followed by NK cells with a "GR response" of ≈50-75%, while monocytes seemed most responsive, with difference in GR expression as high as ≈150%.

**Figure 4.6a-c.** Effect of anxiety proneness on glucocorticoid receptor (GR) expression in selected white blood cell subpopulations (Error bars denote SEM; Abbreviations: MFI, AU – mean fluorescent intensity, arbitrary units)
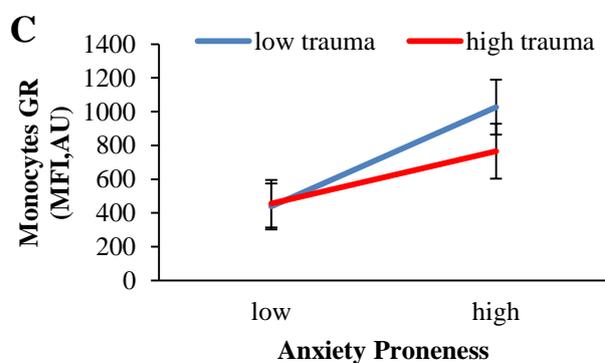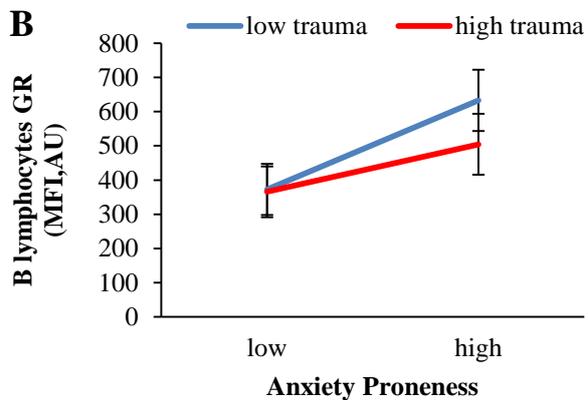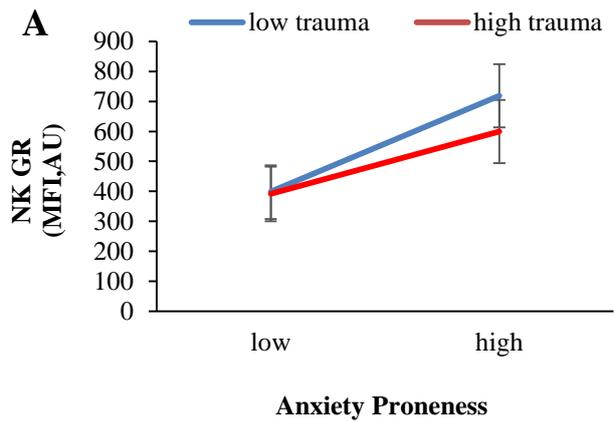
**4.2.4 Circulating cytokine and myeloperoxidase levels**

For serum IL-8, IL-1β, TNF-α, IL-6 and IL-10, the majority of concentrations were below detection limits of the assay kits used. Therefore statistical analysis of these parameters was not feasible. All individuals had valid data for serum IL-12p70 and myeloperoxidase, but neither was affected by either trauma or anxiety proneness (Table 4.4). Looking at the complete dataset, we came to the conclusion that the population as a whole may be chronically immune suppressed, at least in terms of functional capacity, which is why not only IL-8, but also IL-10 was below detectable levels, in contrast to what one might expect. Accordingly, research on normal serum cytokine levels in the adolescent age group revealed medians of 32.6 (28.2-39) pg/mL for IL-8, 11.3 (8.9-13.7) pg/mL for IL-10 and 34.5 (23.2-48.2) pg/mL for IL-12p70 (Kleiner *et al*, 2013). The mean IL-12p70 level for our study population as a whole was $15.4 \pm 19.6$ pg/mL, with a 95% confidence interval range of 9.1-21.8 pg/mL and a median of 8.3 pg/mL. Therefore, in our study population, even for IL-12p70, the only cytokine for which the assay yielded values within the detectable range, levels were much lower compared to the norm. Furthermore, the CBA assay was more sensitive for IL-12p70 than, for example, IL-8 (1.9 pg/ml *versus* 3.6 pg/ml for detection limits, respectively). This could further explain why only this cytokine was detected successfully. However, given the potential general immune suppression in this particular study population, these parameters should be investigated in other adolescent populations as well before firm conclusions may be drawn from this data.

**Table 4.4.** Effect of trauma exposure and anxiety proneness on serum IL-12p70 and MPO

|  | **ANOVA effect of trauma exposure** | **ANOVA effect of anxiety proneness** |
|---|---|---|
| IL-12p70 | p = 0.52 | p = 0.91 |
| MPO | p = 0.7 | p = 0.57 |

**4.3 Central correlates**

**4.3.1 Neurocognitive analysis**

As noted in Chapter 3, a very comprehensive neurocognitive assessment was conducted on the initial 160 participants selected in Phase I, however, this formed part of another project maintaining a psychiatric focus, although running parallel to the current project. For the current study, where the focus was multidisciplinary, we chose a smaller panel of suitable neurocognitive parameters to analyse, in consultation with experienced

research psychologists (Lindi Martin, Sharian Suliman, and Candice Simmons, Department of Psychiatry, Tygerberg Medical School). Due to the time-intensive nature of this particular analysis, results for only 33 participants were available at this point in time, but since they are equally distributed across the spectrum in terms of trauma exposure and anxiety proneness, interpretation of this dataset could be attempted at this stage.

Out of all the neurocognitive variables assessed, only the SSAIS block design total score was influenced by anxiety proneness, with modulation by trauma: There was a main effect of anxiety proneness to decrease SSAIS block design total (ANOVA main effect $P < 0.05$) and trauma exacerbated this effect (for both low and high trauma conditions, anxiety proneness decreased scores, however high trauma in combination with high anxiety proneness was associated with significantly decreased scores (HI-HI vs. LO-LO and LO-HI vs. LO-LO, $P = 0.02$ and $P = 0.07$ respectively)(Fig 4.7a).

The WMS IR overall total score was selected to assess visuospatial working memory as a function allocated to the right hemisphere (SSAIS verbal working memory would be a similar correlate in terms of left hemisphere function). Although there were no main effects of either anxiety proneness or trauma, these measures showed a significant interaction effect (p=0.01, Fig. 4.7b), where anxiety proneness alone resulted in lower visuospatial working memory – an effect reversed in high anxiety prone individuals exposed to trauma.

**Figure 4.7.** Effect of anxiety proneness and trauma on (a) SSAIS block design total score (executive function) and (b) visuospatial (right hemisphere) function (Error bars denote SEM. Abbreviations: SSAIS - Senior South African Individual Scale (revised) test; WMS IR - Wechsler memory scale, Immediate recall score)
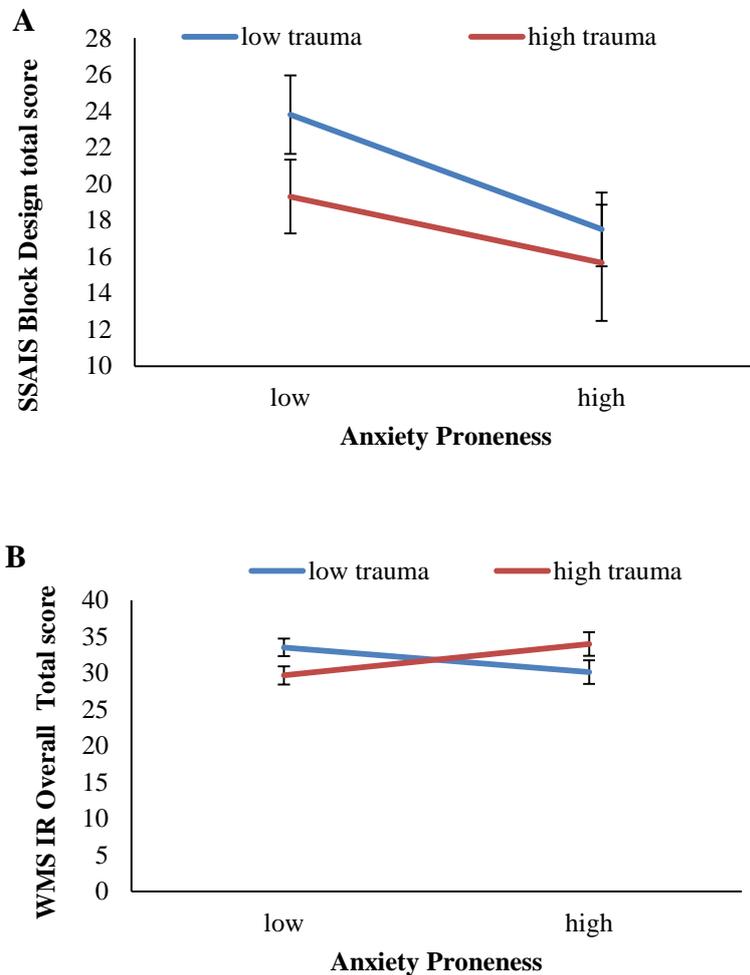


## 4.3.2 Structural MRI analysis

Volumes of the hippocampus, thalamus and PFC did not appear to be significantly affected by either trauma or anxiety proneness in the population studied. However, hemisphere specific sensitivies for these modalities were observed in the amygdala, specifically the right amaygdala. In this context, anxiety proneness and trauma exposure exhibited cumulative additive effects on structural volume. A representative slide to illustrate the technique of measurement is provided in Figure 4.8a, while quantitative data is presented in Figure 4.8b.

**Figure 4.8.** The effect of anxiety proneness to increase grey matter volume in the right amygdala, (a) visually and (b) quantitatively.  For the representative image, a coronal (**A**) and axial (**B**) section taken from Mango ROI imaging software program are shown, to indicate both the right and left amygdala and their position in an MRI T1 weighted image.  (Error bars denote SEM)

**A**



**B**
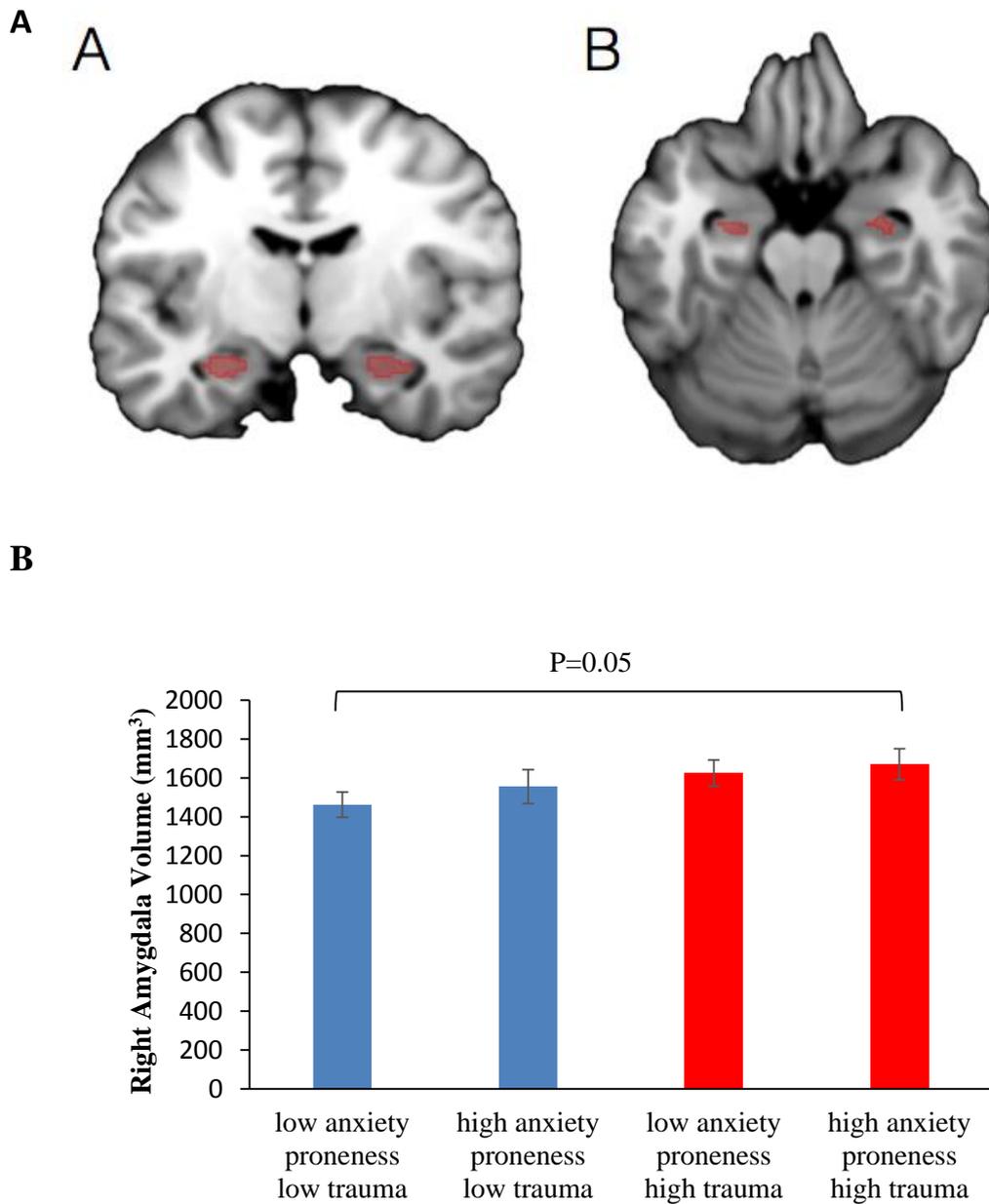


## 4.4 Correlations

We decided to perform correlations between parameters on the whole group, rather than individual groups, for two reasons: firstly, using n=43 would yield more statistical power and secondly, our experimental groups do not represent 4 distinct populations, but rather 4 ranges on one continuum.

**4.4.1 Effects of trauma and anxiety on physiological parameters**

Correlations were calculated between physiological parameters and questionnaires scores, using both total score and sub-scale scores. All correlation results for trait anxiety (STAI-T), anxiety sensitivity (CASI) and trauma exposure (CTQ) are presented in Table 4.5.

**Table 4.5.** Correlations of immune parameters with trait anxiety, anxiety sensitivity (and subscales) and trauma (and subscales)

| Parameter | STAI-T | CASI Social concerns | CASI Psychological concerns | CASI Physical concerns | CASI total | CTQ Physical neglect | CTQ Emotional abuse | CTQ Emotional neglect | CTQ Physical abuse | CTQ Sexual abuse | CTQ total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Total WBC (x 10^9/L) | (-) 0.101 | (-) 0.591 | (-) 0.418 | (-) 0.168 | (-) 0.197 | (+) 0.971 | (+) 0.625 | (-) 0.650 | (-) 0.714 | (+) 0.415 | (+) 0.828 |
| Neutrophils (x 10^9/L) | (-) 0.537 | (+) 0.573 | (+) 0.907 | (-) 0.698 | (-) 0.888 | (+) 0.728 | (+) 0.610 | (+) 0.959 | (+) 0.669 | (+) 0.271 | (+) 0.788 |
| Lymphocytes (x 10^9/L) | **(-) 0.047** | **(-) 0.013** | (-) 0.366 | (-) 0.195 | **(-) 0.063** | (-) 0.528 | (+) 0.805 | (-) 0.374 | (-) 0.737 | (+) 0.642 | (-) 0.693 |
| Monocytes (x 10^9/L) | **(-) <0.01** | **(-) 0.046** | **(-) 0.053** | **(-) <0.01** | **(-) < 0.01** | (+) 0.694 | (-) 0.728 | (+) 0.589 | (-) 0.840 | (-) 0.725 | (-) 0.719 |
| Eosinophils (x 10^9/L) | **(-) <0.01** | (-) 0.349 | (-) 0.138 | (-) 0.096 | (-) 0.087 | **(-) 0.077** | **(-) 0.028** | (-) 0.110 | (-) 0.428 | (-) 0.159 | **(-) 0.089** |
| Basophils (x 10^9/L) | **(-) 0.031** | (-) 0.412 | **(-) 0.039** | **(-) <0.01** | **(-) <0.01** | (+) 0.855 | (+) 0.373 | (+) 0.172 | (-) 0.610 | (+) 0.304 | (+) 0.180 |
| B lymph GR: MFI | (+) 0.135 | (+) 0.972 | (+) 0.443 | **(+) 0.071** | (+) 0.154 | **(-) 0.050** | (+) 0.545 | (-) 0.171 | (-) 0.638 | (-) 0.137 | (-) 0.173 |
| Abs B lymph count | **(-) <0.01** | **(-) <0.01** | (+) 0.293 | (+) 0.100 | **(-) 0.018** | (-) 0.818 | (-) 0.113 | (-) 0.527 | (-) 0.857 | (-) 0.935 | (-) 0.619 |
| NK GR: MFI | (+) 0.156 | (+) 0.948 | (+) 0.413 | **(+) 0.059** | (+) 0.131 | **(-) 0.055** | (+) 0.09 | (-) 0.133 | (+) 0.208 | (-) 0.141 | (-) 0.111 |
| Abs NK cell count | **(-) <0.01** | **(-) 0.016** | (-) 0.416 | **(-) 0.063** | **(-) 0.039** | (-) 0.134 | (-) 0.412 | (-) 0.299 | (-) 0.446 | (-) 0.773 | (-) 0.168 |
| Abs T cell count | (-) 0.214 | (-) 0.542 | (-) 0.244 | **(-) 0.066** | **(-) 0.080** | (-) 0.912 | (+) 0.521 | (+) 0.541 | (+) 0.708 | (+) 0.237 | (-) 0.600 |
| Mono GR: MFI | (+) 0.156 | (+) 0.857 | (+) 0.18 | **(+) 0.094** | (+) 0.122 | (-) 0.222 | (+) 0.512 | (-) 0.149 | (-) 0.369 | (-) 0.297 | (+) 0.242 |
| Prolactin (ug/L) | (-) 0.577 | (-) 0.892 | (-) 0.938 | (-) 0.356 | (-) 0.657 | (-) 0.993 | (-) 0.437 | **(+) 0.044** | (-) 0.555 | (+) 0.172 | (+) 0.405 |
| DHEAS (umol/L) | (-) 0.147 | (-) 0.183 | **(-) 0.021** | **(-) 0.013** | **(-) 0.017** | (-) 0.532 | (+) 0.267 | (-) 0.902 | (-) 0.525 | (-) 0.832 | (-) 0.852 |
| Cortisol (nmol/L) | (-) 0.954 | (+) 0.481 | (-) 0.514 | (-) 0.532 | (-) 0.711 | (+) 0.863 | (-) 0.751 | (+) 0.433 | (-) 0.698 | (+) 0.133 | (+) 0.665 |
| MPO (ng/ml) | (+) 0.614 | (+) 0.169 | (-) 0.488 | (+) 0.954 | (+) 0.792 | (+) 0.952 | **(+) 0.035** | (+) 0.556 | **(+) 0.055** | (+) 0.318 | (+) 0.107 |
| IL-12p70 (pg/mL) | **(+) 0.013** | **(+) <0.01** | (+) 0.149 | (-) 0.271 | **(+) 0.04** | (-) 0.806 | **(+) 0.014** | (+) 0.332 | (+) 0.235 | (-) 0.681 | (+) 0.140 |

Spearman p values are reported, with the nature of correlations in brackets. Significant values and trends are indicated in Bold. Key: WBC: white blood cell, Neu: Neutrophils; Lymph: Lymphocytes; Mono: Monocytes; Eos: Eosinophils; Baso: Basophils; MFI: Mean Fluorescent Intensity; Abs: Absolute

The STAI-T score consistently showed a negative correlation with leukocyte counts, with the exception of neutrophil and total T cell counts, which were not correlated. Neither leukocyte GR expression, nor basal hormone levels were correlated with this parameter. Noteworthy in the context of inflammation was a significant positive correlation between STAI-T and IL-12p70, suggesting that increased trait anxiety was associated with higher pro-inflammatory cytokine status.

CASI total and sub-scale scores showed largely similar correlations as obtained for STAI-T, with a few additional correlations in specific sub-scales. For example, CASI-Total, CASI-Physical concerns and CASI-Psychological concerns were also negatively correlated with basal DHEAs levels (P<0.05), while CASI-Physical concerns showed a consistent tendency for a positive correlation with GR expression for several PBMC types.

In general, CTQ did not show many correlations with physiological parameters. Most notable was a consistent negative correlation of CTQ-Physical neglect with lymphocyte GR expression levels, while CTQ-Emotional abuse showed positive correlations with both serum MPO (P<0.05) and IL-12p70 (P=0.01) levels. CTQ-Physical abuse was also positively correlated with serum MPO (P=0.05), while CTQ-Emotional neglect was positively correlated with basal prolactin level (P<0.05).

**4.4.2 Influences of handedness, resilience and self-esteem on physiological parameters**

Please refer to Table 4.6 for a summary of all correlation p-values.

An interesting finding was that within the normal ranges, total WBC, neutrophil and total T cell counts were negatively correlated with handedness, i.e. a lesser degree of right-handedness in this group was associated with a higher availability of these cells.  In terms of glucocorticoid sensitivity, resilience was positively correlated with GR expression on B lymphocytes.

No correlation was found between any basal hormone level assessed and handedness, resilience or self-esteem, even when considering genders separately.

**Table 4.6.** Correlations of Self-esteem, Resilience, and Handedness with physiological parameters

| Parameter | Rosenberg-SES | CD-RISC | Handedness |
|---|---|---|---|
| Total WBC (x 10^9/L) | (+) 0.598 | (-) 0.741 | **(-) 0.019** |
| Neutrophils (x 10^9/L) | (+) 0.349 | (-) 0.852 | **(-) 0.029** |
| % Neu of total WBC | (+) 0.424 | (-) 0.677 | (-) 0.113 |
| Lymphocytes (x 10^9/L) | (-) 0.940 | (+) 0.846 | (-) 0.260 |
| % Lymph of total WBC | (-) 0.826 | (+) 0.547 | (+) 0.305 |
| Monocytes (x 10^9/L) | (-) 0.433 | (+) 0.430 | (-) 0.379 |
| % Mono of total WBC | (-) 0.220 | (+) 0.309 | (-) 0.990 |
| Eosinophils (x 10^9/L) | (-) 0.252 | **(+) 0.013** | (+) 0.934 |
| % Eos of total WBC | (-) 0.454 | **(+) 0.033** | (+) 0.269 |
| Basophils (x 10^9/L) | (-) 0.813 | (+) 0.400 | (-) 0.672 |
| % Baso of total WBC | (-) 0.779 | (+) 0.407 | (-) 0.800 |
| B lymph GR: Mean Fl Int | (-) 0.599 | **(+) 0.074** | (+) 0.116 |
| Abs B lymph count | (-) 0.197 | (+) 0.360 | (+) 0.415 |
| NK GR: Mean Fl Int | (-) 0.432 | (+) 0.107 | (+) 0.120 |
| Abs NK cell count | (-) 0.689 | (+) 0.177 | (-) 0.116 |
| Abs T cell count | (-) 0.364 | (-) 0.364 | **(-) 0.030** |
| Mono GR: Mean Fl Int | (-) 0.984 | (+) 0.379 | (+) 0.216 |
| Prolactin (ug/L) | (+) 0.16 | (-) 0.24 | (+) 0.93 |
| Testosterone (nmol/L) | (+) 0.98 | (-) 0.46 | (-) 0.80 |
| DHEAs (umol/L) | (+) 0.58 | (+) 0.21 | (-) 0.85 |
| Cortisol (nmol/L) | (-) 0.53 | (-) 0.80 | (-) 0.44 |
| Serum MPO (ng/ml) | (+) 0.595 | (+) 0.550 | (-) 0.579 |
| IL-12p70 (pg/mL) | (+) 0.174 | (+) 0.791 | (+) 0.485 |

Spearman p values are reported and the direction of correlation indicated by either + or -. Significant values and trends are indicated in Bold. Key: Rosenberg-SES: Self-esteem; CD-RISC; Resilience; WBC: white blood cell, Neu: Neutrophils; Lymph: Lymphocytes; Mono: Monocytes; Eos: Eosinophils; Baso: Basophils; Fl Int: Fluorescent intensity; Abs: Absolute

### 4.4.3 Correlation between anxiety and/or trauma with resilience and self-esteem

Since handedness is considered to be a stable trait, no correlation was expected between this parameter and childhood maltreatment which occurs irrespective of cerebral lateralization. Furthermore, no correlation was evident with anxiety proneness as well (data not shown).

In contrast, correlations between both anxiety and trauma, and modulators of adaptive responses (resilience and self-esteem) provided important information. Consistent with the results reported when comparing the 4 experimental groups, trauma (CTQ-total, but also specifically CTQ-Emotional abuse and CTQ-Sexual abuse) showed a positive correlation with self-esteem (R-SES, Table 4.7).  While these results are complex and perhaps more difficult to interpret, the results for resilience were much less so: CTQ-total, as well as CTQ-Physical neglect and CTQ-Physical abuse, showed negative correlations with resilience (CD-RISC, Table 4.7).

**Table 4.7.** Correlation between anxiety and/or trauma with resilience and self-esteem

| Parameter | Self-esteem | Resilience |
|---|---|---|
| **STAI-T** | (+) 0.078 | (-) 0.111 |
| **CASI total** | (+) 0.910 | (+) 0.677 |
| CASI Social Concerns | (+) 0.392 | (+) 0.196 |
| CASI Psychological Concerns | (+) 0.559 | (-) 0.514 |
| CASI Physical Concerns | (-) 0.349 | (+) 0.653 |
| **CTQ total** | **(+) 0.024** | **(-) 0.043** |
| CTQ Physical neglect | (+) 0.251 | **(-) < 0.01** |
| CTQ Emotional abuse | **(+) 0.034** | (-) 0.602 |
| CTQ Emotional neglect | (+) 0.132 | (-) 0.196 |
| CTQ Physical abuse | (+) 0.252 | **(-) 0.070** |
| CTQ Sexual abuse | **(+) 0.091** | (-) 0.262 |

Spearman p values are reported and the direction of correlation indicated by either + or -. Significant values and trends are indicated in Bold.

### 4.4.4 Correlations with central correlates

### 4.4.4.1 Neurocognitive parameters

Since all of the participants were right handed, this indicated a fairly homogenous sample in terms of handedness and therefore cerebral lateralization. This indicated that cerebral dominance with concomitant anatomical asymmetry was not a confounding factor in the analysis of neurocognitive variables.

Preliminary correlations for the 33 participants from whom we have obtained neurocognitive data up to date, are presented in Table 4.8. In terms of anxiety, the STAI-T score was positively correlated with (reverse scored) TOL total time and negatively with SSAIS Block design total, indicating poorer performance associated with trait anxiety (Spearman, P=0.03 and P=0.02 respectively). Furthermore, the CASI subscale of psychological concerns correlated with poorer performance on the SSAIS block design total measure (Spearman, P= 0.04). In the context of trauma, although total trauma score did not statistically affect neurocognitive parameters, again more information was evident when considering the subscales of the CTQ questionnaire:  CTQ physical neglect was correlated with poorer performance on the SSAIS Digit Span Backward total (verbal working memory, Spearman P=0.01) and the CTQ emotional neglect score showed a strong tendency for a positively correlation with executive function as indicated by the TOL total time, indicating pooper executive performance associated with more emotional neglect (Spearman, P=0.05). Furthermore, verbal working memory (as indicated by SSAIS Digit Span Backward total score) was positively related to resilience score (P<0.05).

**Table 4.8.** Correlations between a) STAI-T, b) CASI and c) CTQ and subscales with neurocognitive variables*

| | | Spearman | p-value | n |
|---|---|---|---|---|
| STAIT (Trait anxiety) | WMS IR overall total (Visuospatial Working memory) | -0.09 | 0.62 | 33 |
| STAIT (Trait anxiety) | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.16 | 0.38 | 32 |
| STAIT (Trait anxiety) | SSAIS Block Design total (Executive function) | -0.42 | **0.02** | 32 |
| STAIT (Trait anxiety) | TOL total correct (Executive function) | 0.02 | 0.93 | 29 |
| STAIT (Trait anxiety) | TOL total move score (Executive function) | -0.01 | 0.95 | 27 |
| STAIT (Trait anxiety) | TOL total initiation time (Executive function) | 0.26 | 0.16 | 31 |
| STAIT (Trait anxiety) | TOL total execution time (Executive function) | 0.31 | 0.09 | 31 |
| STAIT (Trait anxiety) | TOL total time (Executive function) | 0.39 | **0.03** | 31 |
| CASI Social concerns | WMS IR overall total (Visuospatial Working memory) | -0.06 | 0.75 | 33 |
| CASI Social concerns | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.36 | 0.05 | 32 |
| CASI Social concerns | SSAIS Block Design total (Executive function) | 0.08 | 0.67 | 32 |
| CASI Social concerns | TOL total correct (Executive function) | 0.16 | 0.39 | 29 |
| CASI Social concerns | TOL total move score (Executive function) | -0.10 | 0.63 | 27 |
| CASI Social concerns | TOL total initiation time (Executive function) | 0.22 | 0.23 | 31 |
| CASI Social concerns | TOL total execution time (Executive function) | -0.08 | 0.68 | 31 |
| CASI Social concerns | TOL total time (Executive function) | 0.08 | 0.66 | 31 |
| CASI Psychological concerns | WMS IR overall total (Visuospatial Working memory) | -0.23 | 0.19 | 33 |
| CASI Psychological concerns | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.24 | 0.19 | 32 |
| CASI Psychological concerns | SSAIS Block Design total (Executive function) | -0.36 | **0.04** | 32 |
| CASI Psychological concerns | TOL total correct (Executive function) | 0.12 | 0.53 | 29 |
| CASI Psychological concerns | TOL total move score (Executive function) | 0.15 | 0.46 | 27 |
| CASI Psychological concerns | TOL total initiation time (Executive function) | 0.03 | 0.85 | 31 |
| CASI Psychological concerns | TOL total execution time (Executive function) | 0.31 | 0.09 | 31 |
| CASI Psychological concerns | TOL total time (Executive function) | 0.26 | 0.15 | 31 |
| CASI Physical concerns | WMS IR overall total (Visuospatial Working memory) | 0.00 | 0.99 | 33 |
| CASI Physical concerns | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.30 | 0.10 | 32 |
| CASI Physical concerns | SSAIS Block Design total (Executive function) | -0.33 | 0.07 | 32 |
| CASI Physical concerns | TOL total correct (Executive function) | 0.03 | 0.89 | 29 |
| CASI Physical concerns | TOL total move score (Executive function) | 0.08 | 0.68 | 27 |
| CASI Physical concerns | TOL total initiation time (Executive function) | 0.12 | 0.51 | 31 |
| CASI Physical concerns | TOL total execution time (Executive function) | 0.22 | 0.24 | 31 |
| CASI Physical concerns | TOL total time (Executive function) | 0.19 | 0.30 | 31 |
| CASI Total | WMS IR overall total (Visuospatial Working memory) | -0.06 | 0.74 | 33 |
| CASI Total | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.35 | 0.05 | 32 |
| CASI Total | SSAIS Block Design total (Executive function) | -0.27 | 0.14 | 32 |
| CASI Total | TOL total correct (Executive function) | 0.10 | 0.60 | 29 |
| CASI Total | TOL total move score (Executive function) | 0.03 | 0.86 | 27 |
| CASI Total | TOL total initiation time (Executive function) | 0.16 | 0.38 | 31 |
| CASI Total | TOL total execution time (Executive function) | 0.18 | 0.32 | 31 |
| CASI Total | TOL total time (Executive function) | 0.20 | 0.27 | 31 |
| CTQ Physical neglect | WMS IR overall total (Visuospatial Working memory) | -0.29 | 0.10 | 33 |
| CTQ Physical neglect | SSAIS Digit Span Backward Total (Verbal Working memory) | -0.43 | **0.01** | 32 |
| CTQ Physical neglect | SSAIS Block Design total (Executive function) | -0.26 | 0.14 | 32 |

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| Table 4.8 (continued) | | Spearman | p-value | n |
|---|---|---|---|---|
| CTQ Physical neglect | TOL total correct (Executive function) | -0.01 | 0.95 | 29 |
| CTQ Physical neglect | TOL total move score (Executive function) | 0.16 | 0.44 | 27 |
| CTQ Physical neglect | TOL total initiation time (Executive function) | 0.01 | 0.98 | 31 |
| CTQ Physical neglect | TOL total execution time (Executive function) | 0.13 | 0.48 | 31 |
| CTQ Physical neglect | TOL total time (Executive function) | 0.05 | 0.78 | 31 |
| CTQ Emotional abuse | WMS IR overall total (Visuospatial Working memory) | 0.06 | 0.76 | 33 |
| CTQ Emotional abuse | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.02 | 0.92 | 32 |
| CTQ Emotional abuse | SSAIS Block Design total (Executive function) | 0.04 | 0.83 | 32 |
| CTQ Emotional abuse | TOL total correct (Executive function) | 0.01 | 0.98 | 29 |
| CTQ Emotional abuse | TOL total move score (Executive function) | -0.15 | 0.46 | 27 |
| CTQ Emotional abuse | TOL total initiation time (Executive function) | 0.08 | 0.68 | 31 |
| CTQ Emotional abuse | TOL total execution time (Executive function) | -0.08 | 0.66 | 31 |
| CTQ Emotional abuse | TOL total time (Executive function) | 0.11 | 0.57 | 31 |
| CTQ Emotional neglect | WMS IR overall total (Visuospatial Working memory) | -0.07 | 0.68 | 33 |
| CTQ Emotional neglect | SSAIS Digit Span Backward Total (Verbal Working memory) | -0.15 | 0.40 | 32 |
| CTQ Emotional neglect | SSAIS Block Design total (Executive function) | -0.20 | 0.28 | 32 |
| CTQ Emotional neglect | TOL total correct (Executive function) | -0.24 | 0.21 | 29 |
| CTQ Emotional neglect | TOL total move score (Executive function) | 0.20 | 0.31 | 27 |
| CTQ Emotional neglect | TOL total initiation time (Executive function) | 0.19 | 0.31 | 31 |
| CTQ Emotional neglect | TOL total execution time (Executive function) | 0.23 | 0.21 | 31 |
| CTQ Emotional neglect | TOL total time (Executive function) | 0.36 | **0.05** | 31 |
| CTQ Physical abuse | WMS IR overall total (Visuospatial Working memory) | -0.08 | 0.68 | 33 |
| CTQ Physical abuse | SSAIS Digit Span Backward Total (Verbal Working memory) | -0.25 | 0.17 | 32 |
| CTQ Physical abuse | SSAIS Block Design total (Executive function) | -0.29 | 0.10 | 32 |
| CTQ Physical abuse | TOL total correct (Executive function) | 0.00 | 0.98 | 29 |
| CTQ Physical abuse | TOL total move score (Executive function) | -0.05 | 0.81 | 27 |
| CTQ Physical abuse | TOL total initiation time (Executive function) | 0.23 | 0.22 | 31 |
| CTQ Physical abuse | TOL total execution time (Executive function) | 0.32 | 0.08 | 31 |
| CTQ Physical abuse | TOL total time (Executive function) | 0.35 | 0.06 | 31 |
| CTQ Sexual abuse | WMS IR overall total (Visuospatial Working memory) | 0.00 | 0.99 | 33 |
| CTQ Sexual abuse | SSAIS Digit Span Backward Total (Verbal Working memory) | -0.08 | 0.65 | 32 |
| CTQ Sexual abuse | SSAIS Block Design total (Executive function) | -0.16 | 0.38 | 32 |
| CTQ Sexual abuse | TOL total correct (Executive function) | -0.36 | 0.06 | 29 |
| CTQ Sexual abuse | TOL total move score (Executive function) | 0.19 | 0.34 | 27 |
| CTQ Sexual abuse | TOL total initiation time (Executive function) | -0.19 | 0.30 | 31 |
| CTQ Sexual abuse | TOL total execution time (Executive function) | 0.00 | 0.98 | 31 |
| CTQ Sexual abuse | TOL total time (Executive function) | 0.00 | 0.99 | 31 |
| CTQ Total | WMS IR overall total (Visuospatial Working memory) | -0.06 | 0.74 | 33 |
| CTQ Total | SSAIS Digit Span Backward Total (Verbal Working memory) | -0.26 | 0.15 | 32 |
| CTQ Total | SSAIS Block Design total (Executive function) | -0.28 | 0.13 | 32 |
| CTQ Total | TOL total correct (Executive function) | -0.06 | 0.75 | 29 |
| CTQ Total | TOL total move score (Executive function) | 0.01 | 0.95 | 27 |
| CTQ Total | TOL total initiation time (Executive function) | 0.20 | 0.29 | 31 |
| CTQ Total | TOL total execution time (Executive function) | 0.16 | 0.40 | 31 |
| CTQ Total | TOL total time (Executive function) | 0.26 | 0.16 | 31 |

*Lower scores indicate better performance for the TOL total move score; TOL total initiation time; TOL total execution time; and TOL total time

Correlations between physiological parameters with neurocognitive functional parameters are presented in Table 4.9. In this study population that had cell counts within normal reference ranges, both higher cell counts and higher GR expression were consistently associated with improved neurocognitive function, with two exceptions: Neutrophil counts and monocyte GR expression were negatively associated with TOL total move score and SSAIS digit span backward total, respectively. Of interest is the fact that the SSAIS digit span backward total score (denoting specifically left hemisphere function), was consistently associated with increased GR expression, with no other neurocognitive parameter correlating with GR expression.

Serum MPO levels were oddly correlated with better functioning indicated by TOL- total correct and –total move scores. Another pro-inflammatory marker, IL-12p70 was also oddly positively correlated with TOL total correct score and negatively correlated with TOL total initiation time (reverse scored). However, given the very low levels reported for other cytokines, this result was difficult to interpret in isolation.

While cortisol showed no direct correlation with any of the neurocognitive parameters assessed, DHEAs levels were positively correlated with two of these measures, namely, SSAIS Block design total score and WMS IR.

**Table 4.9.** Correlations between physiological- (leukocyte counts, leukocyte GR levels and selected circulating protein levels) and neurocognitive variables

| | | Spearman | p-value | n |
|---|---|---|---|---|
| Total WBC (x 10^9/L) | WMS IR overall total (Visuospatial Working memory) | -0.05 | 0.77 | 33 |
| Total WBC (x 10^9/L) | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.10 | 0.57 | 32 |
| Total WBC (x 10^9/L) | SSAIS Block Design total (Executive function) | 0.24 | 0.18 | 32 |
| Total WBC (x 10^9/L) | TOL total correct (Executive function) | -0.09 | 0.64 | 29 |
| Total WBC (x 10^9/L) | TOL total move score (Executive function) | 0.29 | 0.15 | 27 |
| Total WBC (x 10^9/L) | TOL total initiation time (Executive function) | -0.40 | **0.03** | 31 |
| Total WBC (x 10^9/L) | TOL total execution time (Executive function) | -0.13 | 0.49 | 31 |
| Total WBC (x 10^9/L) | TOL total time (Executive function) | -0.13 | 0.49 | 31 |
| Neutrophils (x 10^9/L) | WMS IR overall total (Visuospatial Working memory) | -0.16 | 0.37 | 33 |
| Neutrophils (x 10^9/L) | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.13 | 0.47 | 32 |
| Neutrophils (x 10^9/L) | SSAIS Block Design total (Executive function) | 0.18 | 0.31 | 32 |
| Neutrophils (x 10^9/L) | TOL total correct (Executive function) | -0.07 | 0.72 | 29 |
| Neutrophils (x 10^9/L) | TOL total move score (Executive function) | 0.34 | 0.08 | 27 |
| Neutrophils (x 10^9/L) | TOL total initiation time (Executive function) | -0.26 | 0.16 | 31 |

| Table 4.9 (continued) | | Spearman | p-value | n |
|---|---|---|---|---|
| Neutrophils (x 10^9/L) | TOL total execution time (Executive function) | 0.05 | 0.78 | 31 |
| Neutrophils (x 10^9/L) | TOL total time (Executive function) | 0.07 | 0.71 | 31 |
| Lymphocytes (x 10^9/L) | WMS IR overall total (Visuospatial Working memory) | -0.04 | 0.83 | 33 |
| Lymphocytes (x 10^9/L) | SSAIS Digit Span Backward Total (Verbal Working memory) | -0.05 | 0.77 | 32 |
| Lymphocytes (x 10^9/L) | SSAIS Block Design total (Executive function) | 0.16 | 0.39 | 32 |
| Lymphocytes (x 10^9/L) | TOL total correct (Executive function) | -0.21 | 0.27 | 29 |
| Lymphocytes (x 10^9/L) | TOL total move score (Executive function) | 0.14 | 0.49 | 27 |
| Lymphocytes (x 10^9/L) | TOL total initiation time (Executive function) | -0.31 | 0.09 | 31 |
| Lymphocytes (x 10^9/L) | TOL total execution time (Executive function) | -0.27 | 0.15 | 31 |
| Lymphocytes (x 10^9/L) | TOL total time (Executive function) | -0.32 | 0.07 | 31 |
| Monocytes (x 10^9/L) | WMS IR overall total (Visuospatial Working memory) | 0.26 | 0.15 | 33 |
| Monocytes (x 10^9/L) | SSAIS Digit Span Backward Total (Verbal Working memory) | -0.15 | 0.43 | 32 |
| Monocytes (x 10^9/L) | SSAIS Block Design total (Executive function) | 0.18 | 0.31 | 32 |
| Monocytes (x 10^9/L) | TOL total correct (Executive function) | -0.08 | 0.66 | 29 |
| Monocytes (x 10^9/L) | TOL total move score (Executive function) | 0.11 | 0.59 | 27 |
| Monocytes (x 10^9/L) | TOL total initiation time (Executive function) | -0.13 | 0.50 | 31 |
| Monocytes (x 10^9/L) | TOL total execution time (Executive function) | -0.26 | 0.16 | 31 |
| Monocytes (x 10^9/L) | TOL total time (Executive function) | -0.19 | 0.29 | 31 |
| Eosinophils (x 10^9/L) | WMS IR overall total (Visuospatial Working memory) | 0.18 | 0.32 | 33 |
| Eosinophils (x 10^9/L) | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.18 | 0.32 | 32 |
| Eosinophils (x 10^9/L) | SSAIS Block Design total (Executive function) | 0.26 | 0.14 | 32 |
| Eosinophils (x 10^9/L) | TOL total correct (Executive function) | -0.07 | 0.71 | 29 |
| Eosinophils (x 10^9/L) | TOL total move score (Executive function) | -0.12 | 0.55 | 27 |
| Eosinophils (x 10^9/L) | TOL total initiation time (Executive function) | 0.05 | 0.78 | 31 |
| Eosinophils (x 10^9/L) | TOL total execution time (Executive function) | -0.14 | 0.45 | 31 |
| Eosinophils (x 10^9/L) | TOL total time (Executive function) | -0.01 | 0.95 | 31 |
| Basophils (x 10^9/L) | WMS IR overall total (Visuospatial Working memory) | 0.32 | 0.07 | 33 |
| Basophils (x 10^9/L) | SSAIS Digit Span Backward Total (Verbal Working memory) | -0.21 | 0.24 | 32 |
| Basophils (x 10^9/L) | SSAIS Block Design total (Executive function) | 0.18 | 0.33 | 32 |
| Basophils (x 10^9/L) | TOL total correct (Executive function) | -0.11 | 0.57 | 29 |
| Basophils (x 10^9/L) | TOL total move score (Executive function) | -0.04 | 0.85 | 27 |
| Basophils (x 10^9/L) | TOL total initiation time (Executive function) | -0.01 | 0.95 | 31 |
| Basophils (x 10^9/L) | TOL total execution time (Executive function) | -0.16 | 0.38 | 31 |
| Basophils (x 10^9/L) | TOL total time (Executive function) | -0.10 | 0.59 | 31 |
| Abs B lymph count | WMS IR overall total (Visuospatial Working memory) | 0.11 | 0.56 | 33 |
| Abs B lymph count | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.04 | 0.85 | 32 |
| Abs B lymph count | SSAIS Block Design total (Executive function) | -0.19 | 0.29 | 32 |
| Abs B lymph count | TOL total correct (Executive function) | -0.19 | 0.32 | 29 |
| Abs B lymph count | TOL total move score (Executive function) | 0.20 | 0.31 | 27 |
| Abs B lymph count | TOL total initiation time (Executive function) | -0.44 | **0.01** | 31 |
| Abs B lymph count | TOL total execution time (Executive function) | 0.10 | 0.58 | 31 |
| Abs B lymph count | TOL total time (Executive function) | -0.01 | 0.96 | 31 |
| Abs NK cell count | WMS IR overall total (Visuospatial Working memory) | 0.20 | 0.25 | 33 |
| Abs NK cell count | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.20 | 0.28 | 32 |
| Abs NK cell count | SSAIS Block Design total (Executive function) | 0.07 | 0.68 | 32 |

| Table 4.9 (continued) | | Spearman | p-value | n |
|---|---|---|---|---|
| Abs NK cell count | TOL total correct (Executive function) | -0.27 | 0.15 | 29 |
| Abs NK cell count | TOL total move score (Executive function) | 0.25 | 0.21 | 27 |
| Abs NK cell count | TOL total initiation time (Executive function) | -0.47 | **<0.01** | 31 |
| Abs NK cell count | TOL total execution time (Executive function) | -0.20 | 0.27 | 31 |
| Abs NK cell count | TOL total time (Executive function) | -0.31 | 0.09 | 31 |
| Abs T cell count | WMS IR overall total (Visuospatial Working memory) | 0.31 | **0.08** | 32 |
| Abs T cell count | SSAIS Digit Span Backward Total (Verbal Working memory) | -0.06 | 0.74 | 31 |
| Abs T cell count | SSAIS Block Design total (Executive function) | -0.01 | 0.98 | 31 |
| Abs T cell count | TOL total correct (Executive function) | -0.12 | 0.54 | 28 |
| Abs T cell count | TOL total move score (Executive function) | 0.17 | 0.42 | 26 |
| Abs T cell count | TOL total initiation time (Executive function) | -0.31 | 0.09 | 30 |
| Abs T cell count | TOL total execution time (Executive function) | -0.24 | 0.20 | 30 |
| Abs T cell count | TOL total time (Executive function) | -0.22 | 0.23 | 30 |
| B lymph GR | WMS IR overall total (Visuospatial Working memory) | 0.05 | 0.77 | 33 |
| B lymph GR | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.42 | **0.02** | 32 |
| B lymph GR | SSAIS Block Design total (Executive function) | -0.18 | 0.32 | 32 |
| B lymph GR | TOL total correct (Executive function) | -0.14 | 0.48 | 29 |
| B lymph GR | TOL total move score (Executive function) | -0.14 | 0.49 | 27 |
| B lymph GR | TOL total initiation time (Executive function) | -0.06 | 0.77 | 31 |
| B lymph GR | TOL total execution time (Executive function) | 0.12 | 0.52 | 31 |
| B lymph GR | TOL total time (Executive function) | -0.01 | 0.97 | 31 |
| NK GR | WMS IR overall total (Visuospatial Working memory) | 0.04 | 0.82 | 33 |
| NK GR | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.39 | 0.03 | 32 |
| NK GR | SSAIS Block Design total (Executive function) | -0.19 | 0.30 | 32 |
| NK GR | TOL total correct (Executive function) | -0.08 | 0.70 | 29 |
| NK GR | TOL total move score (Executive function) | -0.15 | 0.44 | 27 |
| NK GR | TOL total initiation time (Executive function) | -0.04 | 0.83 | 31 |
| NK GR | TOL total execution time (Executive function) | 0.14 | 0.44 | 31 |
| NK GR | TOL total time (Executive function) | 0.02 | 0.93 | 31 |
| Mono GR | WMS IR overall total (Visuospatial Working memory) | -0.05 | 0.79 | 33 |
| Mono GR | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.39 | 0.03 | 32 |
| Mono GR | SSAIS Block Design total (Executive function) | -0.11 | 0.55 | 32 |
| Mono GR | TOL total correct (Executive function) | -0.11 | 0.57 | 29 |
| Mono GR | TOL total move score (Executive function) | -0.04 | 0.83 | 27 |
| Mono GR | TOL total initiation time (Executive function) | -0.13 | 0.48 | 31 |
| Mono GR | TOL total execution time (Executive function) | 0.12 | 0.51 | 31 |
| Mono GR | TOL total time (Executive function) | -0.01 | 0.95 | 31 |
| Prolactin (ug/L) | WMS IR overall total (Visuospatial Working memory) | -0.06 | 0.76 | 33 |
| Prolactin (ug/L) | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.13 | 0.48 | 32 |
| Prolactin (ug/L) | SSAIS Block Design total (Executive function) | 0.17 | 0.36 | 32 |
| Prolactin (ug/L) | TOL total correct (Executive function) | -0.01 | 0.94 | 29 |
| Prolactin (ug/L) | TOL total move score (Executive function) | -0.26 | 0.19 | 27 |
| Prolactin (ug/L) | TOL total initiation time (Executive function) | 0.20 | 0.27 | 31 |
| Prolactin (ug/L) | TOL total execution time (Executive function) | -0.08 | 0.68 | 31 |
| Prolactin (ug/L) | TOL total time (Executive function) | 0.12 | 0.52 | 31 |

| Table 4.9 (continued) | | Spearman | p-value | n |
|---|---|---|---|---|
| DHEAs (umol/L) | WMS IR overall total (Visuospatial Working memory) | 0.35 | **0.05** | 33 |
| DHEAs (umol/L) | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.07 | 0.71 | 32 |
| DHEAs (umol/L) | SSAIS Block Design total (Executive function) | 0.50 | **<0.01** | 32 |
| DHEAs (umol/L) | TOL total correct (Executive function) | -0.12 | 0.53 | 29 |
| DHEAs (umol/L) | TOL total move score (Executive function) | -0.22 | 0.27 | 27 |
| DHEAs (umol/L) | TOL total initiation time (Executive function) | 0.22 | 0.23 | 31 |
| DHEAs (umol/L) | TOL total execution time (Executive function) | -0.19 | 0.30 | 31 |
| DHEAs (umol/L) | TOL total time (Executive function) | -0.08 | 0.66 | 31 |
| Cortisol (nmol/L) | WMS IR overall total (Visuospatial Working memory) | 0.03 | 0.86 | 33 |
| Cortisol (nmol/L) | SSAIS Digit Span Backward Total (Verbal Working memory) | -0.07 | 0.70 | 32 |
| Cortisol (nmol/L) | SSAIS Block Design total (Executive function) | 0.21 | 0.24 | 32 |
| Cortisol (nmol/L) | TOL total correct (Executive function) | -0.05 | 0.78 | 29 |
| Cortisol (nmol/L) | TOL total move score (Executive function) | -0.01 | 0.94 | 27 |
| Cortisol (nmol/L) | TOL total initiation time (Executive function) | 0.16 | 0.40 | 31 |
| Cortisol (nmol/L) | TOL total execution time (Executive function) | -0.12 | 0.51 | 31 |
| Cortisol (nmol/L) | TOL total time (Executive function) | -0.01 | 0.96 | 31 |
| Serum MPO (ng/ml) | WMS IR overall total (Visuospatial Working memory) | -0.19 | 0.33 | 30 |
| Serum MPO (ng/ml) | SSAIS Digit Span Backward Total (Verbal Working memory) | -0.26 | 0.17 | 29 |
| Serum MPO (ng/ml) | SSAIS Block Design total (Executive function) | 0.11 | 0.56 | 29 |
| Serum MPO (ng/ml) | TOL total correct (Executive function) | 0.55 | **<0.01** | 26 |
| Serum MPO (ng/ml) | TOL total move score (Executive function) | -0.47 | **0.02** | 24 |
| Serum MPO (ng/ml) | TOL total initiation time (Executive function) | 0.36 | 0.06 | 28 |
| Serum MPO (ng/ml) | TOL total execution time (Executive function) | -0.07 | 0.74 | 28 |
| Serum MPO (ng/ml) | TOL total time (Executive function) | 0.06 | 0.78 | 28 |
| IL-12p70 (pg/mL) | WMS IR overall total (Visuospatial Working memory) | -0.17 | 0.35 | 32 |
| IL-12p70 (pg/mL) | SSAIS Digit Span Backward Total (Verbal Working memory) | 0.05 | 0.78 | 31 |
| IL-12p70 (pg/mL) | SSAIS Block Design total (Executive function) | -0.08 | 0.68 | 31 |
| IL-12p70 (pg/mL) | TOL total correct (Executive function) | 0.39 | **0.04** | 28 |
| IL-12p70 (pg/mL) | TOL total move score (Executive function) | -0.31 | 0.12 | 26 |
| IL-12p70 (pg/mL) | TOL total initiation time (Executive function) | 0.39 | **0.03** | 30 |
| IL-12p70 (pg/mL) | TOL total execution time (Executive function) | -0.04 | 0.82 | 30 |
| IL-12p70 (pg/mL) | TOL total time (Executive function) | 0.21 | 0.28 | 30 |

Lower scores indicate better performance for the TOL total move score; TOL total initiation time; TOL total execution time; and TOL total time.

In terms of potential outcome modulators, neurocognitive parameters did not appear sensitive to change by the ones selected for our purposes. Statistical analysis revealed only a tendency for increased right-handedness to negatively correlate with visuospatial working memory (Spearman -0.31, P=0.08).

**4.4.4.2 Structural MRI parameters**

Similar to the statistical results in terms of group differences, amygdala volume was most prominently correlated positively with parameters associated with anxiety proneness and trauma, and again only in the right hemisphere (Table 4.10). However, in addition, this analysis also revealed a negative correlation between left hemisphere hippocampal volume and CTQ physical abuse. Furthermore, similar to the other seemingly inappropriate response to sexual abuse as trauma, here, right hemisphere hippocampal volume showed a tendency to increase in association with sexual abuse.

**Table 4.10**. Correlations between a) STAI-T, b) CASI and subscales, and c) CTQ and subscales with grey matter volumes

| | | Spearman | p-value | n |
|---|---|---|---|---|
| STAIT (Trait anxiety) | LH Amygdala vol | 0.16 | 0.37 | 34 |
| STAIT (Trait anxiety) | RH Amygdala vol | 0.34 | **0.05** | 34 |
| STAIT (Trait anxiety) | LH Hippo vol | 0.08 | 0.64 | 34 |
| STAIT (Trait anxiety) | RH Hippo vol | 0.15 | 0.40 | 34 |
| STAIT (Trait anxiety) | LH Thalamus vol | 0.18 | 0.30 | 34 |
| STAIT (Trait anxiety) | RH Thalamus vol | 0.15 | 0.41 | 34 |
| STAIT (Trait anxiety) | LH PFC vol | 0.02 | 0.89 | 34 |
| STAIT (Trait anxiety) | RH PFC vol | 0.18 | 0.32 | 34 |
| CASI Social concerns | LH Amygdala vol | -0.01 | 0.97 | 34 |
| CASI Social concerns | RH Amygdala vol | -0.17 | 0.33 | 34 |
| CASI Social concerns | LH Hippo vol | -0.23 | 0.19 | 34 |
| CASI Social concerns | RH Hippo vol | -0.21 | 0.23 | 34 |
| CASI Social concerns | LH Thalamus vol | -0.20 | 0.25 | 34 |
| CASI Social concerns | RH Thalamus vol | -0.06 | 0.72 | 34 |
| CASI Social concerns | LH PFC vol | -0.02 | 0.91 | 34 |
| CASI Social concerns | RH PFC vol | 0.11 | 0.54 | 34 |
| CASI Psychological concerns | LH Amygdala vol | 0.17 | 0.35 | 34 |
| CASI Psychological concerns | RH Amygdala vol | 0.01 | 0.94 | 34 |
| CASI Psychological concerns | LH Hippo vol | 0.06 | 0.74 | 34 |
| CASI Psychological concerns | RH Hippo vol | -0.02 | 0.92 | 34 |
| CASI Psychological concerns | LH Thalamus vol | 0.00 | 0.98 | 34 |
| CASI Psychological concerns | RH Thalamus vol | 0.10 | 0.59 | 34 |
| CASI Psychological concerns | LH PFC vol | -0.15 | 0.39 | 34 |
| CASI Psychological concerns | RH PFC vol | 0.01 | 0.94 | 34 |
| CASI Physical concerns | LH Amygdala vol | 0.19 | 0.28 | 34 |
| CASI Physical concerns | RH Amygdala vol | 0.01 | 0.94 | 34 |
| CASI Physical concerns | LH Hippo vol | -0.02 | 0.90 | 34 |
| CASI Physical concerns | RH Hippo vol | -0.13 | 0.47 | 34 |
| CASI Physical concerns | LH Thalamus vol | -0.06 | 0.74 | 34 |

| Table 4.10 (continued) | | | Spearman | p-value | n |
|---|---|---|---|---|---|
| CASI Physical concerns | RH Thalamus vol | | 0.03 | 0.86 | 34 |
| CASI Physical concerns | LH PFC vol | | -0.10 | 0.58 | 34 |
| CASI Physical concerns | RH PFC vol | | 0.10 | 0.57 | 34 |
| CASI Total | LH Amygdala vol | | 0.16 | 0.36 | 34 |
| CASI Total | RH Amygdala vol | | -0.05 | 0.77 | 34 |
| CASI Total | LH Hippo vol | | -0.04 | 0.80 | 34 |
| CASI Total | RH Hippo vol | | -0.13 | 0.45 | 34 |
| CASI Total | LH Thalamus vol | | -0.09 | 0.62 | 34 |
| CASI Total | RH Thalamus vol | | 0.02 | 0.89 | 34 |
| CASI Total | LH PFC vol | | -0.09 | 0.62 | 34 |
| CASI Total | RH PFC vol | | 0.11 | 0.55 | 34 |
| CTQ Physical neglect | LH Amygdala vol | | 0.12 | 0.50 | 34 |
| CTQ Physical neglect | RH Amygdala vol | | 0.33 | 0.06 | 34 |
| CTQ Physical neglect | LH Hippo vol | | 0.19 | 0.27 | 34 |
| CTQ Physical neglect | RH Hippo vol | | 0.25 | 0.16 | 34 |
| CTQ Physical neglect | LH Thalamus vol | | 0.28 | 0.10 | 34 |
| CTQ Physical neglect | RH Thalamus vol | | 0.27 | 0.12 | 34 |
| CTQ Physical neglect | LH PFC vol | | 0.27 | 0.13 | 34 |
| CTQ Physical neglect | RH PFC vol | | 0.16 | 0.38 | 34 |
| CTQ Emotional abuse | LH Amygdala vol | | 0.08 | 0.64 | 34 |
| CTQ Emotional abuse | RH Amygdala vol | | 0.31 | 0.07 | 34 |
| CTQ Emotional abuse | LH Hippo vol | | 0.20 | 0.26 | 34 |
| CTQ Emotional abuse | RH Hippo vol | | 0.25 | 0.15 | 34 |
| CTQ Emotional abuse | LH Thalamus vol | | 0.26 | 0.15 | 34 |
| CTQ Emotional abuse | RH Thalamus vol | | 0.22 | 0.22 | 34 |
| CTQ Emotional abuse | LH PFC vol | | 0.15 | 0.39 | 34 |
| CTQ Emotional abuse | RH PFC vol | | -0.16 | 0.37 | 34 |
| CTQ Emotional neglect | LH Amygdala vol | | -0.05 | 0.79 | 34 |
| CTQ Emotional neglect | RH Amygdala vol | | 0.25 | 0.15 | 34 |
| CTQ Emotional neglect | LH Hippo vol | | 0.12 | 0.48 | 34 |
| CTQ Emotional neglect | RH Hippo vol | | 0.18 | 0.32 | 34 |
| CTQ Emotional neglect | LH Thalamus vol | | 0.10 | 0.59 | 34 |
| CTQ Emotional neglect | RH Thalamus vol | | 0.21 | 0.24 | 34 |
| CTQ Emotional neglect | LH PFC vol | | 0.05 | 0.76 | 34 |
| CTQ Emotional neglect | RH PFC vol | | -0.09 | 0.62 | 34 |
| CTQ Physical abuse | LH Amygdala vol | | -0.09 | 0.60 | 34 |
| CTQ Physical abuse | RH Amygdala vol | | 0.16 | 0.38 | 34 |
| CTQ Physical abuse | LH Hippo vol | | -0.43 | **0.01** | 34 |
| CTQ Physical abuse | RH Hippo vol | | -0.12 | 0.51 | 34 |
| CTQ Physical abuse | LH Thalamus vol | | -0.22 | 0.21 | 34 |
| CTQ Physical abuse | RH Thalamus vol | | -0.13 | 0.46 | 34 |
| CTQ Physical abuse | LH PFC vol | | 0.08 | 0.65 | 34 |
| CTQ Physical abuse | RH PFC vol | | 0.12 | 0.50 | 34 |
| CTQ Sexual abuse | LH Amygdala vol | | -0.07 | 0.71 | 34 |
| CTQ Sexual abuse | RH Amygdala vol | | 0.29 | 0.10 | 34 |

| Table 4.10 (continued) | | Spearman | p-value | n |
|---|---|---|---|---|
| CTQ Sexual abuse | LH Hippo vol | 0.14 | 0.43 | 34 |
| CTQ Sexual abuse | RH Hippo vol | 0.31 | 0.08 | 34 |
| CTQ Sexual abuse | LH Thalamus vol | -0.06 | 0.74 | 34 |
| CTQ Sexual abuse | RH Thalamus vol | -0.01 | 0.94 | 34 |
| CTQ Sexual abuse | LH PFC vol | -0.09 | 0.60 | 34 |
| CTQ Sexual abuse | RH PFC vol | -0.02 | 0.92 | 34 |
| CTQ Total | LH Amygdala vol | -0.01 | 0.96 | 34 |
| CTQ Total | RH Amygdala vol | 0.41 | **0.01** | 34 |
| CTQ Total | LH Hippo vol | 0.14 | 0.44 | 34 |
| CTQ Total | RH Hippo vol | 0.25 | 0.15 | 34 |
| CTQ Total | LH Thalamus vol | 0.15 | 0.41 | 34 |
| CTQ Total | RH Thalamus vol | 0.24 | 0.17 | 34 |
| CTQ Total | LH PFC vol | 0.16 | 0.38 | 34 |
| CTQ Total | RH PFC vol | -0.05 | 0.79 | 34 |

Abbreviations: LH, left hemisphere; RH, right hemisphere; vol, volume

In terms of leucocyte counts (Table 4.11), a positive correlation was evident between right amygdala volume and absolute T cell count, and between both right- and left-thalamus volumes and absolute NK cell count. In contrast, right PFC hemisphere volume was negatively correlated with lymphocyte count. No correlations between structural volumes and GR expression were evident (Table 4.11).

With regard to hormonal correlations (also Table 4.11), there was a negative correlation between left amygdala volume and prolactin, while the left and right thalamus as well as the left PFC correlated positively with DHEAs.

**Table 4.11**. Correlations between physiological parameters and grey matter volumes

| | | Spearman | p-value | n |
|---|---|---|---|---|
| Total WBC (x 10^9/L) | LH Amygdala vol | 0.23 | 0.19 | 34 |
| Total WBC (x 10^9/L) | RH Amygdala vol | 0.03 | 0.87 | 34 |
| Total WBC (x 10^9/L) | LH Hippo vol | -0.07 | 0.71 | 34 |
| Total WBC (x 10^9/L) | RH Hippo vol | -0.09 | 0.63 | 34 |
| Total WBC (x 10^9/L) | LH Thalamus vol | 0.08 | 0.66 | 34 |
| Total WBC (x 10^9/L) | RH Thalamus vol | 0.25 | 0.16 | 34 |
| Total WBC (x 10^9/L) | LH PFC vol | 0.20 | 0.27 | 34 |
| Total WBC (x 10^9/L) | RH PFC vol | -0.02 | 0.91 | 34 |
| Neutrophils (x 10^9/L) | LH Amygdala vol | 0.17 | 0.35 | 34 |
| Neutrophils (x 10^9/L) | RH Amygdala vol | -0.14 | 0.42 | 34 |
| Neutrophils (x 10^9/L) | LH Hippo vol | -0.15 | 0.40 | 34 |
| Neutrophils (x 10^9/L) | RH Hippo vol | -0.14 | 0.43 | 34 |

| Table 4.11 (continued) | | Spearman | p-value | n |
|---|---|---|---|---|
| Neutrophils (x 10^9/L) | LH Thalamus vol | -0.07 | 0.71 | 34 |
| Neutrophils (x 10^9/L) | RH Thalamus vol | 0.15 | 0.39 | 34 |
| Neutrophils (x 10^9/L) | LH PFC vol | 0.22 | 0.21 | 34 |
| Neutrophils (x 10^9/L) | RH PFC vol | 0.10 | 0.57 | 34 |
| Lymphocytes (x 10^9/L) | LH Amygdala vol | 0.15 | 0.41 | 34 |
| Lymphocytes (x 10^9/L) | RH Amygdala vol | 0.23 | 0.19 | 34 |
| Lymphocytes (x 10^9/L) | LH Hippo vol | 0.09 | 0.62 | 34 |
| Lymphocytes (x 10^9/L) | RH Hippo vol | 0.10 | 0.58 | 34 |
| Lymphocytes (x 10^9/L) | LH Thalamus vol | 0.14 | 0.44 | 34 |
| Lymphocytes (x 10^9/L) | RH Thalamus vol | 0.08 | 0.67 | 34 |
| Lymphocytes (x 10^9/L) | LH PFC vol | -0.04 | 0.81 | 34 |
| Lymphocytes (x 10^9/L) | RH PFC vol | **-0.38** | **0.03** | 34 |
| Monocytes (x 10^9/L) | LH Amygdala vol | 0.01 | 0.95 | 34 |
| Monocytes (x 10^9/L) | RH Amygdala vol | 0.17 | 0.35 | 34 |
| Monocytes (x 10^9/L) | LH Hippo vol | 0.01 | 0.94 | 34 |
| Monocytes (x 10^9/L) | RH Hippo vol | -0.01 | 0.97 | 34 |
| Monocytes (x 10^9/L) | LH Thalamus vol | 0.18 | 0.31 | 34 |
| Monocytes (x 10^9/L) | RH Thalamus vol | 0.23 | 0.19 | 34 |
| Monocytes (x 10^9/L) | LH PFC vol | 0.05 | 0.78 | 34 |
| Monocytes (x 10^9/L) | RH PFC vol | -0.08 | 0.67 | 34 |
| Eosinophils (x 10^9/L) | LH Amygdala vol | -0.16 | 0.36 | 34 |
| Eosinophils (x 10^9/L) | RH Amygdala vol | -0.21 | 0.24 | 34 |
| Eosinophils (x 10^9/L) | LH Hippo vol | **-0.33** | **0.05** | 34 |
| Eosinophils (x 10^9/L) | RH Hippo vol | -0.28 | 0.11 | 34 |
| Eosinophils (x 10^9/L) | LH Thalamus vol | -0.19 | 0.28 | 34 |
| Eosinophils (x 10^9/L) | RH Thalamus vol | -0.15 | 0.41 | 34 |
| Eosinophils (x 10^9/L) | LH PFC vol | 0.07 | 0.71 | 34 |
| Eosinophils (x 10^9/L) | RH PFC vol | -0.01 | 0.96 | 34 |
| Basophils (x 10^9/L) | LH Amygdala vol | -0.12 | 0.52 | 34 |
| Basophils (x 10^9/L) | RH Amygdala vol | 0.20 | 0.26 | 34 |
| Basophils (x 10^9/L) | LH Hippo vol | 0.11 | 0.52 | 34 |
| Basophils (x 10^9/L) | RH Hippo vol | 0.09 | 0.60 | 34 |
| Basophils (x 10^9/L) | LH Thalamus vol | -0.13 | 0.48 | 34 |
| Basophils (x 10^9/L) | RH Thalamus vol | -0.04 | 0.82 | 34 |
| Basophils (x 10^9/L) | LH PFC vol | -0.09 | 0.63 | 34 |
| Basophils (x 10^9/L) | RH PFC vol | -0.21 | 0.24 | 34 |
| B lymph GR: Mean Fl Int | LH Amygdala vol | -0.17 | 0.34 | 34 |
| B lymph GR: Mean Fl Int | RH Amygdala vol | -0.10 | 0.58 | 34 |
| B lymph GR: Mean Fl Int | LH Hippo vol | -0.03 | 0.85 | 34 |
| B lymph GR: Mean Fl Int | RH Hippo vol | -0.13 | 0.45 | 34 |
| B lymph GR: Mean Fl Int | LH Thalamus vol | 0.13 | 0.45 | 34 |
| B lymph GR: Mean Fl Int | RH Thalamus vol | -0.11 | 0.55 | 34 |
| B lymph GR: Mean Fl Int | LH PFC vol | -0.01 | 0.94 | 34 |
| B lymph GR: Mean Fl Int | RH PFC vol | 0.01 | 0.96 | 34 |
| Abs B lymph count | LH Amygdala vol | -0.07 | 0.71 | 34 |

| Table 4.11 (continued) | | Spearman | p-value | n |
|---|---|---|---|---|
| Abs B lymph count | RH Amygdala vol | 0.27 | 0.13 | 34 |
| Abs B lymph count | LH Hippo vol | -0.07 | 0.69 | 34 |
| Abs B lymph count | RH Hippo vol | -0.26 | 0.14 | 34 |
| Abs B lymph count | LH Thalamus vol | 0.08 | 0.66 | 34 |
| Abs B lymph count | RH Thalamus vol | 0.07 | 0.69 | 34 |
| Abs B lymph count | LH PFC vol | -0.13 | 0.47 | 34 |
| Abs B lymph count | RH PFC vol | 0.00 | 1.00 | 34 |
| NK GR: Mean Fl Int | LH Amygdala vol | -0.10 | 0.59 | 34 |
| NK GR: Mean Fl Int | RH Amygdala vol | -0.09 | 0.62 | 34 |
| NK GR: Mean Fl Int | LH Hippo vol | 0.06 | 0.75 | 34 |
| NK GR: Mean Fl Int | RH Hippo vol | -0.07 | 0.71 | 34 |
| NK GR: Mean Fl Int | LH Thalamus vol | 0.18 | 0.31 | 34 |
| NK GR: Mean Fl Int | RH Thalamus vol | -0.05 | 0.77 | 34 |
| NK GR: Mean Fl Int | LH PFC vol | -0.02 | 0.90 | 34 |
| NK GR: Mean Fl Int | RH PFC vol | 0.01 | 0.97 | 34 |
| Abs NK cell count | LH Amygdala vol | 0.17 | 0.33 | 34 |
| Abs NK cell count | RH Amygdala vol | 0.18 | 0.30 | 34 |
| Abs NK cell count | LH Hippo vol | 0.08 | 0.65 | 34 |
| Abs NK cell count | RH Hippo vol | 0.17 | 0.34 | 34 |
| Abs NK cell count | LH Thalamus vol | 0.31 | 0.07 | 34 |
| Abs NK cell count | RH Thalamus vol | **0.38** | **0.03** | 34 |
| Abs NK cell count | LH PFC vol | 0.00 | 1.00 | 34 |
| Abs NK cell count | RH PFC vol | -0.15 | 0.39 | 34 |
| Abs T cell count | LH Amygdala vol | 0.13 | 0.46 | 33 |
| Abs T cell count | RH Amygdala vol | **0.41** | **0.02** | 33 |
| Abs T cell count | LH Hippo vol | 0.06 | 0.75 | 33 |
| Abs T cell count | RH Hippo vol | 0.03 | 0.86 | 33 |
| Abs T cell count | LH Thalamus vol | -0.16 | 0.39 | 33 |
| Abs T cell count | RH Thalamus vol | -0.02 | 0.93 | 33 |
| Abs T cell count | LH PFC vol | -0.08 | 0.68 | 33 |
| Abs T cell count | RH PFC vol | -0.22 | 0.23 | 33 |
| Mono GR: Mean Fl Int | LH Amygdala vol | -0.03 | 0.86 | 34 |
| Mono GR: Mean Fl Int | RH Amygdala vol | -0.15 | 0.39 | 34 |
| Mono GR: Mean Fl Int | LH Hippo vol | 0.06 | 0.75 | 34 |
| Mono GR: Mean Fl Int | RH Hippo vol | -0.03 | 0.89 | 34 |
| Mono GR: Mean Fl Int | LH Thalamus vol | 0.25 | 0.15 | 34 |
| Mono GR: Mean Fl Int | RH Thalamus vol | 0.08 | 0.67 | 34 |
| Mono GR: Mean Fl Int | LH PFC vol | 0.11 | 0.54 | 34 |
| Mono GR: Mean Fl Int | RH PFC vol | 0.03 | 0.85 | 34 |
| Prolactin (ug/L) | LH Amygdala vol | **-0.39** | **0.02** | 34 |
| Prolactin (ug/L) | RH Amygdala vol | -0.33 | 0.06 | 34 |
| Prolactin (ug/L) | LH Hippo vol | 0.03 | 0.89 | 34 |
| Prolactin (ug/L) | RH Hippo vol | -0.07 | 0.69 | 34 |
| Prolactin (ug/L) | LH Thalamus vol | -0.16 | 0.36 | 34 |
| Prolactin (ug/L) | RH Thalamus vol | -0.16 | 0.36 | 34 |

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| Table 4.11 (continued) | | Spearman | p-value | n |
|---|---|---|---|---|
| Prolactin (ug/L) | LH PFC vol | 0.01 | 0.93 | 34 |
| Prolactin (ug/L) | RH PFC vol | 0.03 | 0.87 | 34 |
| DHEAs (umol/L) | LH Amygdala vol | 0.09 | 0.60 | 34 |
| DHEAs (umol/L) | RH Amygdala vol | 0.01 | 0.94 | 34 |
| DHEAs (umol/L) | LH Hippo vol | 0.05 | 0.76 | 34 |
| DHEAs (umol/L) | RH Hippo vol | 0.25 | 0.15 | 34 |
| DHEAs (umol/L) | LH Thalamus vol | **0.38** | **0.03** | 34 |
| DHEAs (umol/L) | RH Thalamus vol | 0.33 | 0.06 | 34 |
| DHEAs (umol/L) | LH PFC vol | **0.36** | **0.04** | 34 |
| DHEAs (umol/L) | RH PFC vol | 0.01 | 0.94 | 34 |
| Cortisol (nmol/L) | LH Amygdala vol | -0.14 | 0.42 | 34 |
| Cortisol (nmol/L) | RH Amygdala vol | -0.08 | 0.64 | 34 |
| Cortisol (nmol/L) | LH Hippo vol | 0.10 | 0.59 | 34 |
| Cortisol (nmol/L) | RH Hippo vol | 0.00 | 1.00 | 34 |
| Cortisol (nmol/L) | LH Thalamus vol | -0.05 | 0.77 | 34 |
| Cortisol (nmol/L) | RH Thalamus vol | -0.04 | 0.83 | 34 |
| Cortisol (nmol/L) | LH PFC vol | -0.02 | 0.89 | 34 |
| Cortisol (nmol/L) | RH PFC vol | -0.08 | 0.65 | 34 |
| Serum MPO (ng/ml) | LH Amygdala vol | 0.15 | 0.44 | 30 |
| Serum MPO (ng/ml) | RH Amygdala vol | -0.09 | 0.64 | 30 |
| Serum MPO (ng/ml) | LH Hippo vol | -0.22 | 0.25 | 30 |
| Serum MPO (ng/ml) | RH Hippo vol | -0.14 | 0.47 | 30 |
| Serum MPO (ng/ml) | LH Thalamus vol | -0.08 | 0.68 | 30 |
| Serum MPO (ng/ml) | RH Thalamus vol | -0.04 | 0.82 | 30 |
| Serum MPO (ng/ml) | LH PFC vol | 0.11 | 0.58 | 30 |
| Serum MPO (ng/ml) | RH PFC vol | 0.04 | 0.84 | 30 |
| IL-12p70 (pg/mL) | LH Amygdala vol | 0.12 | 0.52 | 32 |
| IL-12p70 (pg/mL) | RH Amygdala vol | -0.04 | 0.81 | 32 |
| IL-12p70 (pg/mL) | LH Hippo vol | 0.01 | 0.97 | 32 |
| IL-12p70 (pg/mL) | RH Hippo vol | -0.03 | 0.86 | 32 |
| IL-12p70 (pg/mL) | LH Thalamus vol | -0.19 | 0.29 | 32 |
| IL-12p70 (pg/mL) | RH Thalamus vol | -0.14 | 0.45 | 32 |
| IL-12p70 (pg/mL) | LH PFC vol | -0.08 | 0.68 | 32 |
| IL-12p70 (pg/mL) | RH PFC vol | -0.25 | 0.16 | 32 |

Abbreviations: LH, left hemisphere; RH, right hemisphere; vol, volume

Furthermore, in terms of the potential outcome modifying factors assessed (Table 4.12), only one correlation was statistically significant: left hemisphere PFC volume was positively correlated with self-esteem.

**Table 4.12.** Correlations between self-esteem, resilience and handedness and grey matter volumes

|  |  | Spearman | p-value | n |
|---|---|---|---|---|
| Self-esteem (RSES) | LH Amygdala vol | -0.05 | 0.80 | 34 |
| Self-esteem (RSES) | RH Amygdala vol | -0.11 | 0.52 | 34 |
| Self-esteem (RSES) | LH Hippo vol | -0.06 | 0.74 | 34 |
| Self-esteem (RSES) | RH Hippo vol | 0.22 | 0.21 | 34 |
| Self-esteem (RSES) | LH Thalamus vol | 0.03 | 0.86 | 34 |
| Self-esteem (RSES) | RH Thalamus vol | 0.04 | 0.82 | 34 |
| Self-esteem (RSES) | LH PFC vol | **0.35** | **0.04** | 34 |
| Self-esteem (RSES) | RH PFC vol | 0.16 | 0.37 | 34 |
| Resilience (CD-RISC) | LH Amygdala vol | -0.16 | 0.37 | 34 |
| Resilience (CD-RISC) | RH Amygdala vol | -0.17 | 0.32 | 34 |
| Resilience (CD-RISC) | LH Hippo vol | -0.16 | 0.36 | 34 |
| Resilience (CD-RISC) | RH Hippo vol | -0.18 | 0.30 | 34 |
| Resilience (CD-RISC) | LH Thalamus vol | -0.11 | 0.54 | 34 |
| Resilience (CD-RISC) | RH Thalamus vol | -0.08 | 0.65 | 34 |
| Resilience (CD-RISC) | LH PFC vol | 0.08 | 0.67 | 34 |
| Resilience (CD-RISC) | RH PFC vol | 0.00 | 1.00 | 34 |
| Handedness (EHI) | LH Amygdala vol | -0.15 | 0.41 | 34 |
| Handedness (EHI) | RH Amygdala vol | -0.26 | 0.14 | 34 |
| Handedness (EHI) | LH Hippo vol | 0.03 | 0.87 | 34 |
| Handedness (EHI) | RH Hippo vol | -0.24 | 0.16 | 34 |
| Handedness (EHI) | LH Thalamus vol | -0.03 | 0.85 | 34 |
| Handedness (EHI) | RH Thalamus vol | -0.18 | 0.31 | 34 |
| Handedness (EHI) | LH PFC vol | -0.20 | 0.26 | 34 |
| Handedness (EHI) | RH PFC vol | -0.18 | 0.30 | 34 |

Abbreviations: LH, left hemisphere; RH, right hemisphere; vol, volume

## 4.5 Betamethasone suppression and Trier Social Stress Test

For the majority of samples, ACTH was below detectable level (<0.2 pmol/l; normal range 1.6 - 13.9 pmol/L), indicating sufficient suppression of ACTH secretion by the administered betamethasone, which was maintained up to the post-TSST time point. For 4 individuals (in various groups), ACTH at pre-TSST was not suppressed. These individuals were excluded from further analysis on the assumption of non-compliance in taking their betamethasone the evening before.

Unfortunately, for the population as a whole, both pre- and post-TSST cortisol levels were very low (29.8±5.9nmol/L and 32.7±6.4nmol/L respectively), suggesting that the inhibition may have been too severe for this population to overcome, so that we cannot accurately interpret the cortisol response to the TSST.

Turning attention to purely psychological assessments, the state version of the State/Trait Anxiety Inventory (STAI-S) was assessed only once, prior to the TSST, i.e. during HPA-suppression with betamethasone. Both trauma and anxiety proneness seemed to decrease scores for this parameter to some extent (ANOVA main effects for trauma and anxiety proneness $P<0.05$ and $P=0.08$ respectively), with HI-HI exhibiting the lowest STAI-S score and LO-LO the highest. STAI-S was similar for both low trauma groups, and also similar for the two high trauma groups. (Fig.4.9).

**Figure 4.9.** The effect of anxiety proneness and trauma on state anxiety (Error bars denote SEM. Abbreviations: STAI-state - State-Trait Anxiety Inventory, state version)
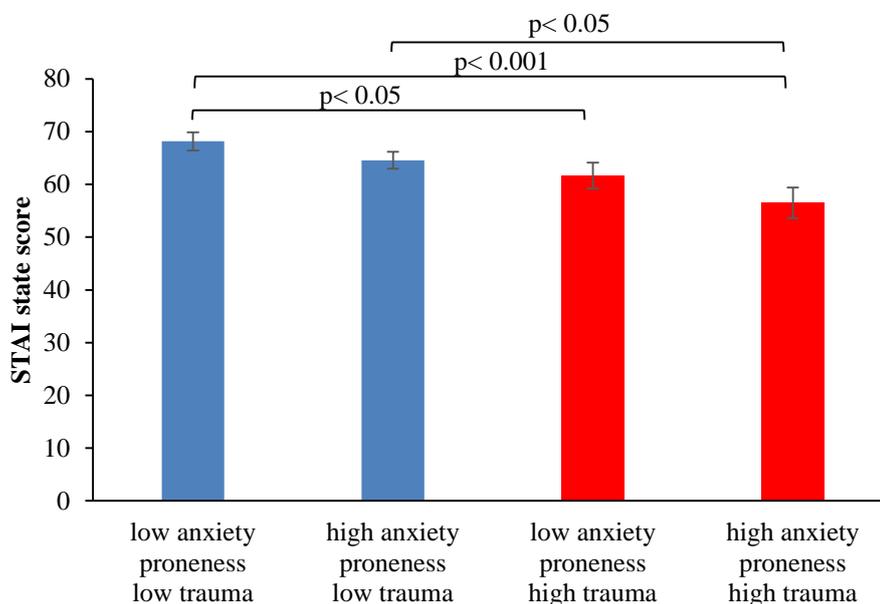


Subjective perception of state anxiety level as measured by the VAS for anxiety was assessed pre- and post- of the TSST. The VAS score for the group increased significantly in response to the TSST (ANOVA main effect; $P<0.00001$), validating the efficacy of the TSST to induce psychosocial stress. VAS scores for anxiety were not influenced by trauma (ANOVA effect of trauma not significant, $P=0.395$), while anxiety proneness seemed to increase VAS scores at baseline only (Fig. 4.10). Interestingly, while low anxiety proneness was associated with significantly lower pre-TSST VAS score, post-TSST VAS scores were similarly high for both high and low anxiety proneness (Fig. 4.10).

**Figure 4.10.** The effect of trauma exposure and anxiety proneness on VAS (anxiety) scores (Error bars denote SEM. Abbreviations: VAS TSST - Visual Analogue Scale for anxiety, pre-and post-Trier Social Stress Test).
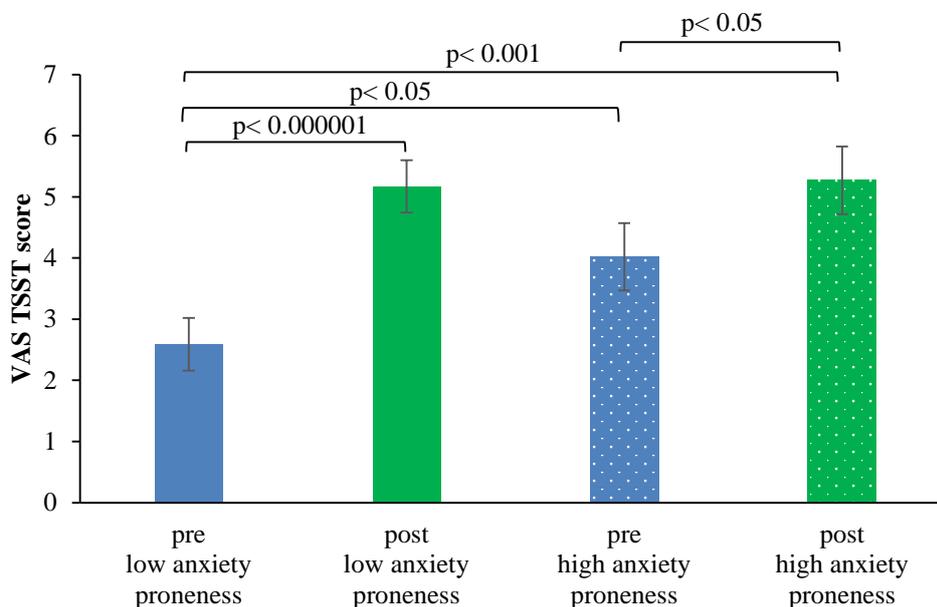


In terms of correlations to OMFs, while experimentally induced acute psychosocial stress (TSST) outcome was not affected by self-esteem or resilience as assessed in the current study, handedness did seem to affect the outcome: both pre- and post TSST, handedness was positively correlated with cortisol and VAS (perception of level of anxiety) (Table 4.13), indicating that a higher degree of consistent right-handedness was associated with more perceived state anxiety.

**Table 4.13.** Correlations between self-esteem, resilience and handedness and perceived anxiety level

|  |  | **Spearman** | **p-value** | **Cases** |
|---|---|---|---|---|
| Self-esteem (RSES) | VAS Pre-TSST | 0.03 | 0.86 | 41 |
| Self-esteem (RSES) | VAS Post-TSST | -0.10 | 0.54 | 41 |
| Resilience (CD-RISC) | VAS Pre-TSST | -0.08 | 0.64 | 41 |
| Resilience (CD-RISC) | VAS Post-TSST | -0.02 | 0.89 | 41 |
| Handedness (EHI) | VAS Pre-TSST | **0.37** | **0.02** | 41 |
| Handedness (EHI) | VAS Post-TSST | **0.28** | **0.08** | 41 |

# CHAPTER 5

# DISCUSSION

By simultaneously assessing both physiological and psychological parameters, the current study was able to point out a significant oversight in the literature. The literature clearly shows very different profiles for two conditions that are characterized by a) hyperactivation of the hypothalamus, enhanced negative feedback, and counterregulatory adaptation of the adrenal gland (hypocortisolism), compared to b) hypoactivation of the hypothalamus, decreased negative feedback and consequent hyperreactivity of the adrenal gland. Although in both conditions, high, normal or low cortisol levels have been reported, the former scenario generally involves increased CRH and/or GR expression levels and enhanced Dex suppression (denoting enhanced negative central feedback). This has been observed in PTSD (De Kloet, et al., 2007; Griffin, et al., 2005; Heim, et al., 2000; van Zuiden, et al., 2013; Yehuda, et al., 1991) and melancholia (Gold & Chrousos, 2002; Tsigos & Chrousos, 2002), where the construct of anxiety underlies both disorders (Winter, 2009; Zoellner, et al., 2011). In contrast, the latter condition is signified by decreased CRH and/or GR expression levels as well as Dex suppression being overridden (denoting impaired negative central feedback) and has been associated with chronic stress (not burn-out) and major or atypical depression (Brown, et al., 2004; Checkley, 1996; Herman, et al., 1995; Holsboer, 2000; Martin, et al., 2009; Mizoguchi, et al., 2003; Pariante & Miller, 2001; Pruessner, et al., 1999; Pruessner, et al., 2003; van der Knaap, et al., 2014; Zunszain, et al., 2011). However, despite these contrasting physiological findings, the confounding effect of anxiety has largely been ignored in studies on trauma exposure – such as childhood maltreatment – where the majority of reports did not consider anxiety levels of individuals (e.g. (Carpenter, et al., 2007; Cicchetti, et al., 2010; MacMillan, et al., 2009; Newport, et al., 2004). We have shown that, in a population exposed to chronic traumatic stress (childhood maltreatment), the level of trauma had a relatively insignificant role to play in central information processing and physiological outcome parameters, when compared to the predictive power of anxiety proneness (impetus for viewing anxiety proneness as a predictor is provided in section 5.1). This major, novel result highlights the importance of assessing childhood trauma in relation to anxiety.

Interpretation of data combining multiple disciplines has its challenges: in psychology, large subject numbers are the norm, while in physiological studies usually much smaller subject numbers are required for sufficient statistical power. Due to financial constraints, the combined assessment of psychological and physiological parameters was carried out in a smaller subgroup of the total recruited population. Thus, we have approached

interpretation of data very conservatively – only effects consistently evident were used to draw final conclusions from these data so as to avoid over-interpretation. In order to place the results reported into context, we would like to highlight the following novel aspects of our investigation:

a) The current study is the first to have investigated psycho-neurophysiological profiles in adolescents of low socio-demographic background, but outside the context of PTSD or depression diagnosis (or diagnosis of any other mental disorder).

b) It is the first study to have considered the effects of childhood trauma in combination with anxiety proneness, by investigating the relative contributions of each of these two modalities to neurophysiological maladaptations.

c) It is the first study to have comprehensively explored the brain-body link by simultaneous investigation of psychological and physiological parameters, both centrally and peripherally.

d) It is the first study to have identified specific markers which can be used in early diagnosis of risk to develop an anxiety disorder, in a population with high incidence of exposure to moderate to high levels of complex trauma.

e) It is the first study to have administered a psychosocial stress challenge in combination with betamethasone suppression, in adolescents.

In the next sections, the main results will be discussed in context with available literature. This will be followed by a conclusion on new potential diagnostic markers useful in this context, as well as suggestions for therapeutic intervention and further research.

**5.1 Validation of appropriate subject selection**

We were able to confirm that the CTQ questionnaire which was used as a measure of childhood maltreatment, as well as the two questionnaires which were used in combination as an indication of anxiety proneness (STAI-T and CASI), have been validated by means of statistical analysis. Interestingly, in addition to trait anxiety being associated with anxiety proneness (as expected), trait anxiety was also higher with higher trauma exposure levels. Trait anxiety is considered to be both a trait and a personality dimension - the term trait implying a generalized and enduring predisposition to react to many situations in a consistent manner (Allport, 1937) and personality dimensions denoting moderate heritability (Loehlin, 1992). Although the current study was of cross-sectional nature – in the absence of intervention and follow-up measures, causation cannot be determined – we

could safely assume that we have measured psycho-neurophysiological effects caused by anxiety proneness and that the neurophysiological determinants did not give rise to anxiety proneness. This is based on the above mentioned heritability aspect associated with anxiety proneness, suggesting that neurophysiological underpinnings associated with anxiety proneness signify responses to anxiety proneness, since anxiety proneness is established during infancy and the neurophysiological make-up of an individual is only fully developed/matured in late adolescence. Furthermore, considering the numerous accounts of activation of the inflammatory response system in depression (reviewed in Schiepers et al, 2005) and in models of anxiety/trauma (reviewed in section 2.4.5), in our opinion, if causality constituted the direction of systemic aberrations resulting in psychiatric disorders, it would have been possible to reverse the development of these conditions *via* anti-inflammatory medication. Therefore, in the current thesis, we have regarded associations with anxiety proneness to be effects thereof.

Consequently, given our results in a cross-section of one generation, which showed significant effects of anxiety proneness but not trauma, the association between current trauma and trait anxiety suggested that anxiety as a personality trait may have been transferred across a number of generations in populations subjected to enduring traumatizing living conditions. The growing body of literature suggesting transgenerational transfer of epigenetic modulation in response to various stimuli (e.g., (Harper, 2005; Sharma, 2013; Szyf, 2015), supports this interpretation. This implied that perhaps the effects of trauma cannot be accurately measured by assessment of one generation only and that measures of trait anxiety and anxiety sensitivity, shown to have a hereditary component (Legrand, et al., 1999; Stein, et al., 1999), indicated levels of anxiety proneness as a  function of both anxiety-associated genes as well as transgenerational epigenetic imprints related to traumatic circumstances.

**5.2 Relative contributions of anxiety proneness *versus* childhood maltreatment to clinical phenotype**

We investigated the relative effects of trauma and anxiety proneness on a) physiological parameters, b) central correlates and c) markers indicating potential outcome modulators (self-esteem, handedness and resilience). This was done in light of our anxiety-associated information processing model in Chapter 2 (fig 2. section 2.1.1), so as to investigate the interplay of nature *versus* nurture on pathological stimulus processing and to highlight which avenues i.e., nature, nurture, physiology, or the interface of these three areas, are the most appropriate to target with diagnosis and/or intervention strategies.

Main findings on the associations/effects of anxiety proneness and trauma included:

a. In terms of basal endocrine parameters, ANOVA showed a main effect of anxiety proneness (with no effect of trauma) to decrease early morning rested serum levels of cortisol and DHEAs.

b. In terms of the basal immune profile, independently of trauma, high anxiety proneness was generally associated with a lower count in pro-inflammatory cell types, such as monocyte counts.

c. In terms of basal GR levels, while statistical analysis revealed an effect of anxiety proneness to increase GR expression on various subpopulations of leukocytes, closer inspection revealed that trauma seemed to consistently dampen this effect across leukocyte subpopulations. However, again we point out that these results were obtained by manually gating for the CD45+ population and without the required standardisation, so that future studies should test the validity of this conclusion.

d. In terms of neurocognitive data, anxiety proneness and trauma exposure exhibited cumulative additive negative effects on executive function, as indicated by the SSAIS block design total score.

e. In terms of structural MRI data, anxiety proneness and trauma exposure exhibited cumulative additive effects on right amygdala volume.

These respective effects of anxiety proneness and the phenomenon of trauma modulating the effects of anxiety proneness in some instances, may be explained by exploring different conceptual frameworks of information processing. Although there exists ample evidence for anxiety being associated with an enhanced tendency to detect negativity in ambiguous stimuli that may signify the presence of threat (e.g. (Huppert, et al., 2007), there is evidence that contextual information overrules this bias (e.g., (Blanchette & Richards, 2003)). We will therefore assess the results of our study, mentioned in the preceding paragraph, in relation to these two different perspectives on information processing. This is in relation to our findings signifying (mal)adaptations to pathological stimuli processing, where we considered the particular style of stimuli processing to be a dispositional characteristic. However, before this can be undertaken, it is important to clarify certain concepts that come into play. The first is the concept of contextual information, where in relation to the current study, long term contextual information may represent exposure to either high or low levels of trauma over a period of years, where adaptation to trauma has set in. In the case of individuals being exposed to high levels of trauma over a number of years, their contextual information framework is generally characterized by high levels of threat posed by the environment, with these individuals frequently being immersed in high risk/damage situations.

The second concept to clarify is that of mood-congruency. Mood has been described as longer lasting than emotion, being diffuse, non-specific and a broad dimension, characterized by either positive affect or negative affect (Ekkekakis, 2013). Mood congruence is defined as the concordance between a person's affective state and the valence of the stimulus (Gilboa-Schechtman, et al., 2000). Trait anxiety, a higher order personality dimension, has been associated with mood-congruent effects on stimulus interpretation (Blanchette, et al., 2007). Support has been provided for individuals scoring high on the personality dimension of trait anxiety to also present with the overall propensity to experience negative mood and anxiety. This is based on research suggesting that measures of neuroticism or negative affectivity (of which the STAI-T is a major indicator) a) are more predictive of levels of state affect (mood) across situations and are less reactive to specific stressors (Rapee & Medoro, 1994) and b) are associated with the tendency to experience negative emotions such as depression, anger, and anxiety (Vuoskoski & Eerola, 2011). This provides support for the mediation approach where personality traits predispose individuals to certain mood states, which then influence emotional processing (Rusting, 1998). Furthermore, evidence has been provided for personality traits, such as neuroticism, and mood states to interact, thereby influencing mood-congruent cognitive judgment (Rusting, 1999).

We now move on to apply two hypotheses involving the processing of ambiguous stimuli to our findings. One hypothesis is the mood-congruency hypothesis (e.g. (Bower, 1981), where, in the context of the current study, mood-congruent stimulus processing in high anxiety prone individuals, refers to an overall inclination for information processing to be affected by negative mood, with the latter denoting a clinical feature of these individuals. Thus, the mood-congruent hypothesis implies that ambiguity is resolved in the context of the individual's mood with the external context having no influence on the direction of the interpretation. More specifically, regardless of whether the individual has or is continuing to be exposed to high levels of maltreatment, a high anxiety-prone individual would interpret a threat/neutral ambiguous stimulus in a threatening manner, whereas a non-anxious person would resolve this ambiguity in the neutral direction. Furthermore, the high anxiety prone individual will present with psycho-neurophysiological (mal)adaptations as a results of this style of information processing, whether he/she has been exposed to trauma or not. This hypothesis in is line with our findings of a main effect of anxiety proneness on cortisol, DHEAs, and monocyte counts, and no effect of trauma. To date, aside from investigation by Blanchette and Richards of the mood-congruency hypothesis in the context of anxiety (summarized in (Blanchette & Richards, 2010), support for the mood-congruency hypothesis has mostly been provided in the context of depression (e.g., (Hertel & El-Messidi, 2006; Miller & Norman, 1986; Wisco & Nolen-Hoeksema, 2010)) and to the best of our knowledge, no other

study has investigated the mood-congruency hypothesis in relation to outcome in physiological parameters. Therefore, the current study is the first to provide support for the mood-congruency hypothesis in the context of information processing in anxiety and in relation to physiological outcomes.

In contrast, the context-sensitivity hypothesis (Blanchette & Richards, 2003) predicts context congruent resolutions. In other words, when the context is negative such is the case with high trauma exposure, the ambiguity in the ambiguous stimulus will be resolved in the mood-congruent direction. For example, in the case of high trauma exposed individuals, if current mood is also negative as is predominantly the case with high anxiety prone individuals, ambiguous stimuli will be resolved in a negative way. This was evident in the exaggeration of the effects of anxiety proneness on total lymphocyte counts with high levels of trauma as well as the cumulative additive effects of anxiety proneness and trauma on SSAIS block design total scores and amygdala volume. However, when that same stimulus is resolved within a positive or neutral context, the resolution will be mood-incongruent (i.e. not affected by anxiety proneness). Thus, in our study's context, at low levels of trauma, stimulus resolution would not be influenced by anxiety proneness. Although this scenario might have been true for total lymphocyte counts - where the main effect of anxiety proneness to reduce counts was not evident in the low trauma exposed individuals-, the low trauma exposed individuals have still been exposed to trauma, albeit not high levels, and therefore the context in which stimulus resolution takes place cannot be classified as "positive or neutral". Also, researchers investigating the context-sensitivity hypothesis have manipulated relatively short-term context (Aviezer, et al., 2008; Barrett, et al., 2007; Lucas, 1999), by, for example, submitting participants to prolonged exposure to either a series of positive/neutral facial expressions or a series fearful expressions in advance of an emotional facial expression processing task (Butler, et al., 2008; Richards, et al., 2013). Therefore, these types of investigations do not allow for comparison with our study in the context of low levels of trauma exposure where it is not possible to extrapolate low levels of trauma to that of a positive/neutral context. Thus, the context-sensitivity hypothesis cannot be applied in the interpretation of our data at low levels of trauma.

In the current study, only self-esteem and resilience were affected by trauma relatively independent of anxiety proneness (especially when correlations with the STAI-T, CASI and CTQ were also considered). In our opinion, although we have not statistically assessed for moderating influences by these factors, these were potential modulating factors of information processing styles and not outcome variables of information processing such as the case for the neurophysiological parameters assessed. Therefore, in terms of the mood-congruent and

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context-sensitive hypotheses, our assessment of neurophysiological outcome measures as downstream products of information processing most powerfully supported the mood-congruent hypothesis. However, a potential avenue for further investigation of the context-sensitivity hypothesis thus remains: if a positive context could be provided by intervention, the validity of this hypothesis could be addressed in the current population with more accuracy. To summarize, information processing with downstream physiological sequelae was influenced by anxiety proneness (nature) to a greater extent than trauma (nurture), although trauma exposure did seem to modulate the effects of anxiety proneness with some of the parameters assessed. We have modified Figure 2.2 to include the current study's results and to more clearly indicate our understanding and/or opinions (Fig 5.1).

**Figure 5.1.** Relative contributions of anxiety proneness and childhood maltreatment to clinical outcome in adolescents, as well as modulation by self-esteem, handedness and resilience
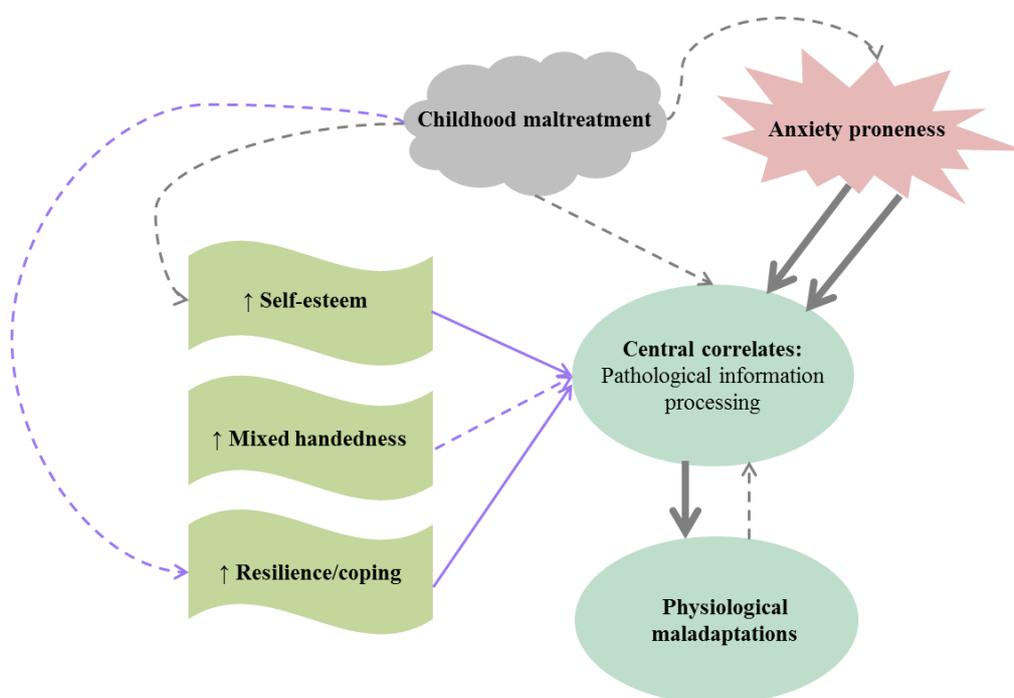


**Key:** Solid arrows: consistent effect; dashed arrow: limited effect; purple arrows: diminishing effect; grey arrow: enhanced effect.

As evident from fig 5.1, our results indicated a relatively larger contribution of anxiety proneness than maltreatment in measures underlying pathological information processing and subsequent physiological sequelae. However, independently of anxiety proneness, childhood maltreatment was associated with increased self-esteem and decreased resilience, and these potential modulating factors, in turn, exhibited favorable effects on neurophysiological outcome. Another potential modulating factor we suggested to impact

neurophysiological parameters in a favorable manner was that of handedness, or more specifically, increased inconsistent (mixed) handedness.

In the next four sections, a more detailed discussion of the relative effects of anxiety proneness and trauma on specific outcome measures follows.

### 5.2.1 DHEAs – a potential marker for HPA responsivity

An analysis of basal serum hormone levels revealed the following:

In terms of cortisol, as is evident from representative review articles, cortisol has commonly been assessed in the context of chronic stress and allostasis (Karatsoreos & McEwen, 2011), and in models of depression (O'Keane, et al., 2012) and PTSD (Morris, et al., 2012), but not in populations presenting with subclinical anxiety. Furthermore, DHEAs has mostly been investigated in models of ageing (Samaras, et al., 2013) and PTSD (see section Chapter 2, section 2.5.2). Therefore, we highlight the novelty of the current study's finding involving anxiety proneness to have uniquely decreased basal levels of both cortisol (a trend) and DHEAs in subclinical anxiety, independently of trauma. Considering the trajectory from hyper- to hypocortisolism reviewed in Chapter 2, this, together with the fact that in our study population, DHEAs levels were below normal for the majority of the participants - which is a possible indicator of adrenal burnout/allostasis - may point towards hypoactivity of the lower (excluding the hypothalamus) HPA-axis, dependent on anxiety proneness and which was already evident in the current study's age group. The fact that anxiety proneness showed to significantly affect DHEAs levels and only a trend was evident in the case of cortisol, in addition to more central correlations with DHEAs (executive function and visuospatial working memory, as well as thalamus and left PFC volumes, refer to Tables 4.9 and 4.11), compared to no correlations with cortisol, suggested that DHEAs is a more consistent marker than cortisol to indicate longer-term chronic adaptations to, for example, anxiety proneness. This might have been the case because DHEAs does not have a potentially confounding metabolic role, such as in the case of cortisol. Thus, DHEAs is a potentially useful marker in monitoring progressive changes in HPA-axis sensitivity over years, and an indirect marker of cortisol responsivity.

The overall low level of DHEA evident across all four groups in this population might have been due to other factors, such as nutrition, in addition to the effect of anxiety proneness in the high anxiety groups. This also highlights that regardless of anxiety proneness- and trauma exposure- level, this population was at risk for neurophysiological aberrations associated with allostasis (Pfaua & Russo, 2015), even at a relatively young age

already. This, together with the abnormally low cytokine levels observed across the group as a whole, suggested that our study population was a vulnerable group, even in absence of social confounders such as the ones addressed in the study.

In terms of testosterone, given the well-known and understood gender differences for testosterone levels in the context of disorders of childhood and adolescence (reviewed in (Zahn-Waxler, et al., 2008)), testosterone data should be interpreted in a gender dependent manner. We considered the number of males in the study (n = 11) as insufficient to accurately interpret their testosterone data in the context of trauma and anxiety proneness. When considering the females (n = 32), testosterone concentration did not seem to be influenced by either anxiety proneness or trauma. Of course, given the relatively low basal level of this hormone in females, it does not exclude a role for this hormone in adolescent males (Granger, et al., 2003). This thus remains a viable avenue for further study.

### 5.2.2 Leukocyte counts

The fact that all leukocyte cell counts across the study population were within normal ranges, indicated that none of the participants presented with severe pathologically challenged immune systems at the time of sample and data collection, although the very low cytokine levels observed may indicate relatively blunted immune activity.

As mentioned already, trauma exposure had relatively little influence in terms of immune cell count, especially when interpreted in comparison to the effects ascribed to anxiety proneness. High anxiety prone groups exhibited lower leukocyte counts of all subtypes with the exception of neutrophils, B and NK cells. According to the literature, increases in cortisol levels (Buckingham, et al., 1996) as well as psychological disorders such as depression and schizophrenia which are associated with chronically raised cortisol levels  (Kronfol, et al., 1984), have been associated with a loss of lymphocytes and mononuclear cells from circulation, whereas neutrophil numbers increase in circulation. This increased neutrophil:lymphocyte ratio may be dependent on the chronicity of stress *via* the action of cortisol (Davis, et al., 2008). Therefore, a first interpretation of the decreased cells counts (excluding neutrophils) involves the adaptation, i.e. lower peripheral cell number, to the initial chronic condition of raised cortisol levels (HPA-axis hyperactivity) in high anxiety prone individuals, with this adaptation still being conserved under conditions of "early blunting" of the lower HPA-axis. This is based on our data which exhibited a trend for anxiety proneness (considered to be a trait and therefore exerting an enduring effect) to decrease basal cortisol levels, in effect pointing towards initiation of hypoactivity of the

lower HPA-axis in terms of the postulated long term trajectory from hyper- to hypoactivity of the lower HPA-axis.

A second interpretation involves the possible effect of acute stress on leukocytes in circulation. In rats, acute stress has been accompanied by increasing magnitudes of peripheral blood lymphocyte trafficking to the skin and this stress-induced redeployment of lymphocytes was attenuated with increasing exposure to chronic stress and correlated with attenuated glucocorticoid responsivity (Dhabhar & McEwen, 1997). Therefore, in our population, in the high anxiety prone individuals, there might have been a loss in circulating lymphocytes and monocytes in response to an acute increase in cortisol levels in anticipation of the blood draw.

A third interpretation suggests cortisol independent mechanisms responsible for the decreases in lymphocyte and monocyte counts observed. For example, evidence in rodent models suggests that catecholamines play an important role in the redistribution of blood leukocytes during more acute social stress (a social confrontation procedure) (Engler, et al., 2004). In humans, chronic stress has been showed to decrease B lymphocytes, T-suppressor/cytotoxic lymphocytes, and natural killer cells and resulted in greater numbers of neutrophils, which were positively correlated with epinephrine levels (McKinnon, et al., 1989). However, this effect associated with catecholamines is unlikely, given the fact that our data showed no significant decreases in B- and NK cell counts associated with either anxiety proneness or trauma exposure.

Indeed, it was proven a difficult task to interpret data in the context of available literature, simply because no literature is available on this topic. Even in the context of PTSD, immune outcome has not been the focus of research. Furthermore, changes in numbers and percentages of leukocyte subpopulations may be due to diurnal fluctuations as well as the presence or absence of mild acute stress conditions: in rats, peripheral blood leukocyte counts varied with time of day and these variations were similar to changes observed under mild acute stress conditions (Dhabhar, et al., 1994). Therefore, in our opinion, using peripheral leukocyte counts in diagnosis of anxiety proneness should at best be accompanied by a measure which is relatively more stable across the time of day, such as leukocyte GR expression. We will therefore move on to an assessment of GR expression in conjunction with leukocyte counts in the next section.

### 5.2.3 Increased glucocorticoid sensitivity

Since studies on the regulation of GRs in the human brain are not feasible, indirect approaches have been followed by assessing white blood cell (WBC) GR expression, representing GR expression in the body at large

(Calfa, et al., 2003). In the current study, GR expression was consistently increased with anxiety proneness, in all of the WBC subpopulations assessed. (This included both NKT- and T cell populations that were excluded from individual interpretation (refer to section 4.2.2).)  This provided support for the general assumption that WBC GR expression is an indication of uniform and overall up or down regulation of GR expression in most, if not all cell types. Indeed, in order to obtain a measurement reflecting GR expression throughout the body (both centrally and peripherally), GR expression has generally been assessed in PBMCs in most studies. Therefore, a discussion follows on GR expression in the context of leukocyte counts.

In all leukocyte subpopulations assessed for GR expression, there was a major effect of anxiety proneness to increase GR expression. This observed enhanced mononuclear leukocyte glucocorticoid responsiveness/ sensitivity is in line with studies on PTSD (see (c) in section 2.4.5.1). We acknowledge once again the fact that our GR assessment was flawed according to current quality control guidelines, and by no means advocate not adhering to the standardization protocols prescribed. However, the fact that our GR data are in accordance with the anxiety literature, albeit in the context of a more extreme case of anxiety – PTSD – and since this result was consistently present in all WBC subpopulations assessed, in our opinion, the relevance of this finding, and thus the importance of assessing GR levels as indicator of GC sensitivity, cannot be disputed. Our finding was also unique in the sense that it contrasted with the literature on models of chronic stress, where GR is generally down-regulated (e.g. (Webster & Cidlowski, 2006)). Since in our study, trauma seemed to blunt the anxiety proneness-induced increase in GR expression consistently across all leukocyte sub-populations assessed, this may suggest that with chronic stress or childhood maltreatment, GR expression may be down-regulated in response to chronically raised cortisol levels.

However, with anxiety proneness, potential hypo-activation of the HPA-axis (as was observed in the current population) may result in decreased basal cortisol levels and a compensatory increase in GR expression so as to increase sensitivity to cortisol. Since we also observed consistent decreases in peripheral leukocyte counts associated with anxiety proneness, this may reflect a possible detrimental effect of chronically upregulated GR levels, due to cortisol-induced apoptosis, particularly in the case of lymphocytes. As evident from many original articles, (e.g. (Krüger, et al., 2011; Purton, et al., 2004)) and summarized in numerous reviews (e.g. (Distelhorst, 2002; Planey & Litwack, 2000; Schaaf & Cidlowski, 2002), cortisol induces apoptosis in certain cells of the lymphoid lineage, particularly immature thymocytes as well as mature lymphocytes and this type of cell death is initiated by, and strictly dependent upon the interaction of cortisol with the GR (Hala, et al., 1996; Helmberg, et

al., 1995). However, evidence support the need for GR to be sufficiently expressed and maintained for a considerable time, in order to exert its apoptotic function (Brunet, et al., 1998) and in cells sensitive to the cytolytic effect of GC, *in vitro* evidence for GR auto-induction has been provided (Gomi, et al., 1990; Kofler, et al., 2003; Pedersen & Vedeckis, 2003; Ramdas, et al., 1999). Although there was a tendency for anxiety proneness to decrease cortisol levels, levels of cortisol in the current population were still within the normal range. Therefore, the significantly increased GR expression in the high anxiety prone individuals may reflect GR auto-induction in response to *relatively* low cortisol levels, with subsequent increased cortisol sensitivity in turn having led to apoptosis of leukocytes (mainly lymphocytes).  Furthermore, although increased GR expression in response to acute stress or immune system activation may be beneficial in that it would enhance cortisol's anti-inflammatory actions, chronically increased GR expression in association with normal cortisol levels may present with detrimental side-effects, such as increased apoptosis of immune cells. Indeed, as mentioned above, anxiety proneness was shown to decrease peripheral blood leukocytes, along with increasing GR expression. Naturally, with increased GR expression, one would assume greater cortisol sensitivity, along with more cortisol-induced anti-inflammatory effects. However, this did not seem to be the case.

The fact that all of the leukocyte counts assessed, showed a consistent negative correlation with trait anxiety, with the exception of neutrophils, together with the fact that IL-12p70 concentration - promoting a shift towards Th1 immunity (Vieira, et al., 1998) - was positively correlated with trait anxiety, suggests that higher trait anxiety promoted a pro-inflammatory phenotype characterized by relatively higher neutrophil counts and higher levels of serum IL-12p70. This assumption is strengthened by the fact that anxiety sensitivity was similarly correlated with leukocyte counts (B- and T-cells, monocytes, basophils, and NK cells) and IL-12p70, in addition to a negative correlation with DHEAs (refer to Table 4.5), where low levels of DHEAs might have also contribute to a pro-inflammatory phenotype (Chen & Parker, 2004; Russo, et al., 2012). Furthermore, the physical concerns subscale of the anxiety sensitivity subscale was also negatively correlated with DHEAs, along with a consistent tendency for a positive correlation with GR expression in the entire leukocyte population assessed.

The latter results might be attributed to the anxiety prone individuals' immune systems that have been less able to mount acute GR-mediated responses, since the capacity for GR to be further up-regulated acutely had been diminished due to chronically up-regulated GR expression. Therefore, under conditions of acute stress, there was a diminished capacity for cortisol to exert anti-inflammatory effects, with higher levels of these markers of

inflammation maintained. Taken together, the unique effects of anxiety proneness on GR expression may render GR analysis an efficient tool in diagnostics, especially given the fact that GR expression is a relatively stable marker across a 24-hour time period. These effects of increased GR expression along with decreased leukocyte counts and increased systemic inflammation may be unique to anxiety and signify a profile not apparent under conditions of childhood maltreatment. Accordingly, given our results of anxiety-prone associated increases in GR expression (which appeared to be blunted by trauma exposure) and studies showing increased GR expression in PTSD (Rohleder, et al., 2004; Yehuda, 2001; Yehuda, et al., 2004), increased glucocorticoid sensitivity may be a unique function of anxiety and not trauma exposure. Therefore, we suggest that trauma-focused literature (e.g. on PTSD and childhood maltreatment), up to date be revisited, so as to assess whether effects previously ascribed to trauma may in fact be due to effects of anxiety proneness.

More specifically, compared to the correlations observed in our population in terms of trait anxiety and anxiety sensitivity with leukocyte counts, the total trauma score did not correlate with any peripheral blood cell counts. In addition, an opposite direction of association to that observed with anxiety sensitivity, was evident with the physical neglect trauma subscale: in contrast to the anxiety sensitivity physical concern subscale, physical neglect was consistently negatively correlated with lymphocyte GR expression levels. However, as was the case for specific measures of anxiety, emotional abuse and physical abuse were positively correlated with markers of inflammation, i.e., serum MPO and IL-12p70 levels (refer to Table 4.5). Surprisingly, no significant correlations were observed for sexual abuse.

### 5.2.4 Top-down information processing

Both anxiety proneness and trauma exposure influenced neurocognitive outcomes associated with prefrontal cortex (PFC) executive function, and this was more apparent when the participant cohort as a whole was used in determining correlations between neurocognitive parameters and the STAI-T, CASI and CTQ: executive function which is associated with the PFC, correlated negatively with the anxiety scales, (STAI-T and CASI) and poorer functioning of the PFC and specifically the left PFC was associated with emotional neglect and physical neglect, respectively. This notion of poorer executive function linked to neglect and not abuse, has also been observed in recent research by other groups (Nikulina & Widom, 2013; Revington, et al., 2011) although these studies did not account for the possible confounding effects of anxiety on these measures. Interestingly, when correlations between these scales and structural volumes were considered, trauma was again uniquely

associated with the left hemisphere, although in this case physical abuse also correlated with decreased left hippocampal volume.

In terms of left *versus* right hemisphere neurocognitive function, given the evidence for a modulating role of anxiety in disrupting specifically functions of visuospatial working memory attributed to the right PFC (and not verbal working memory, associated with the left PFC) (Shackman, et al., 2006), one might expect anxiety proneness to be associated with poorer visuospatial working memory function as indicated by the WMS IR score. However, when group comparisons were made in the current study, there was no main effect of anxiety proneness on visuospatial working memory as a function of the right PFC and only an interaction effect between anxiety proneness and trauma was evident.  This effect may be due to individual differences in right hemisphere functioning as a result of anxious arousal elicited during the testing procedure, in both trauma and anxiety groups, since relatively poorer right PFC function and more activity in this region has been suggested to be a unique function of anxious arousal as opposed to anxious apprehension (trait anxiety) (Nitschke, et al., 1999; Shackman, et al., 2006). Furthermore, considering this interaction effect, unlike the research by Shackman and colleagues, trauma (and not anxiety) resulted in lower visuospatial working memory which was reversed in high anxiety prone individuals exposed to trauma. This highlights the need for future studies to investigate further the links between anxiety *versus* trauma on neurocognitive function, associated with left *versus* right hemisphere dominance.

Since deficits in PFC function also influence functioning in other brain areas, and considering the functional connectivity between the PFC and the amygdala, we will discuss our findings of synergistic effects of anxiety proneness and trauma on right amygdala volume and the aforementioned neurocognitive results, in unison. Although it is not possible to directly compare data obtained from structural *versus* functional MRI scans, we provide and account of the potential processes underling information processing, as assessed by both structural and functional means. Evidence for the interrelatedness of the PFC and amygdala is provided from functional studies on emotion processing, where, for example, the amygdala has been shown to tightly functionally couple to the PFC, and this relationship has been suggested as important for several neurocognitive domains, including emotion regulation, stimulus evaluation, visceral responses, and attentional processing (Banks, et al., 2007; Bzdok, et al., 2012; Robinson, et al., 2010). Accordingly, reduced functional connectivity between the medial PFC and the amygdala has been implicated in youth with PTSD (Wolf & Herringa, 2015) and relatively greater right prefrontal cortical activation in favour of the left when negative affective emotional stimuli are presented,

has been associated with less effective inhibition of the right amygdala and reduced fear extinction (Amaral, et al., 1992; Gewirtz, et al., 1997; Morgan, et al., 1993). Furthermore, EEG coherence have indicated that compared to healthy controls, maltreated children showed right hemispheres that were significantly more developed than their left (Teicher, et al., 1997). These studies collectively indicate that under conditions of anxiety and/or maltreatment, there is less inhibition of the amygdala by the PFC, specifically the right amygdala. Therefore, our findings of compromised PFC functioning along with increased right amygdala volume provided support for this notion. Furthermore, given that in the current study, anxiety proneness was associated with increased GR expression as well as increased right amygdala volume (potentially indicating long-term hyperactivity in this area), and that evidence exists for GR expression to predict amygdala activity in PTSD (Geuze, et al., 2012), GR expression level may be used in monitoring emotional information processing in high anxiety prone individuals.

However, research considering specifically amygdala volumetric abnormalities associated with anxiety and childhood maltreatment is sparse. In contrast to our finding of increased right amygdala volume in high anxiety prone individuals, one study reported reduced amygdala volumes in pediatric patients with anxiety disorder (Milham, et al., 2005) and another found decreased amygdala volumes in healthy children with histories of early life stress (i.e., physical abuse, early neglect, or low socioeconomic status) (Hanson, et al., 2015).  However, Milham's group assessed amygdala volume in children who have been diagnosed with an anxiety disorder and Hanson's group found decreased amygdala volume to be associated with childhood trauma (where anxiety proneness was not assessed and might have had an opposite effect to that of trauma). In addition, these inconsistencies in the literature may be due to confounding factors impacting gray matter measures, such as gender (Wilke, et al., 2007), genetics (Meyer-Lindenberg, et al., 2006) and volumetry method (Doty, et al., 2008). In line with the current study however, even in healthy children as young as ages 7 to 9, high childhood anxiety has been associated with enlarged amygdala volume (Qin, et al., 2014). Considering the lack of research on this topic as well as the inconsistencies in the existing literature, the need is highlighted for more studies to be done, specifically investigating amygdala volume (both left and right) in association with anxiety proneness, compared to trauma exposure.

Nevertheless, given our results of compromised PFC neurocognitive function, along with increased right amygdala volume in high anxiety prone individuals (exacerbated by trauma), we postulated that reduced executive functioning leaded to less effective inhibition of the right amygdala during emotional processing. This

then, in turn, might have resulted in frequent states of hyperreactivity of the right amygdala, which over time, might have led to increased right amygdala volume. Given that in the current study, larger right amygdala volume was associated with anxiety prone individuals, this may indicate a scenario of more acquisition of fear and less extinction of fear conditioning in these individuals, seeing that amygdala hyperactivity has been implicated in fear processing (Goossens, et al., 2007) and has been associated with amygdala hypertrophy (Hölzel, et al., 2009; McEwen, 2003). Furthermore, since these measures were compounded by trauma exposure, this may point towards the effects of frequent traumatic and fear-provoking situations instigating even more fear conditioning. We therefore further postulate that over the course of years, this hyperactivity of the right amygdala every time negative (or even just ambiguous) stimuli are presented, may result in structural changes in this region, denoted by the observed increased volumes in this region.

Indeed, although only presented in functional studies, amygdala hyper-reactivity to emotion processing tasks has been frequently observed in patients presenting with PTSD, panic disorder and generalized anxiety disorder (Rauch, et al., 1996a; Shin, et al., 2005b; Taylor, et al., 2007) as well as in high-anxious individuals (Abraham, et al., 2013; Laeger, et al., 2012; Sehlmeyer, et al., 2011) and individuals displaying anxious attachment styles (Vrticka, et al., 2008). This was also the case in healthy adults with a history of childhood maltreatment (Dannlowski, et al., 2012) as well as anxiety prone participants (maltreatment history is unknown), although anxiety proneness (as a function of neuroticism, trait anxiety, and anxiety sensitivity) was associated with greater activation of predominantly the left amygdala (Stein, et al., 2007). Even though to our knowledge, no study has addressed the issue of increased amygdala hyperreactivity with concomitant increased amygdala volume, one study revealed such an association in healthy adults with separation anxiety (Redlich, et al., 2014). This finding is relevant to the current study since separation anxiety has been proposed to create a strong vulnerability for a number of affective and anxiety disorders (Lewinsohn, et al., 2008; Silove, et al., 2010) and shares common features with other domains of anxiety (Bögels, et al., 2013).

Our study is the first to have reported increased right amygdala volume – which may denote either a pre-existing predisposition or an acquired function of long-term maladaptation - as well as poorer PFC functioning, where both of these outcomes were associated with anxiety proneness, and exaggerated by trauma. The compromised PFC neurocognitive functioning observed in the current study was an indication of suboptimal information processing in the absence of emotionally valanced stimuli. This, in our opinion, reflected a compromised neurocognitive attributional style predisposing information processing under most conditions of stimulus

presentation, not only under emotion-provoking conditions. Again, as was discussed in the case of cumulative effects of trauma on right amygdala volume, this style of information processing could lead to increased (McEwen, 2003), in our case increased right amygdala volume, possibly reflecting maladaptation to frequent bouts of amygdala hyperreactivity due to a selection bias for and interpretation of negative stimuli, over the course of years. Taken together, this scenario denoted less effective emotional (subcortical) control by the PFC. The fact that this maladaptation on a structural level was already evident in adolescents, further highlights the necessity to therapeutically target at-risk populations from a young age.

### 5.2.4.1 Information processing associated with resilience

Given evidence for a) the PFC exerting cognitive control over emotions (e.g. (Miller & Cohen, 2001; Ochsnera & Gross, 2005; van Honk & Schutter, 2006)), specifically control over negative emotions *via* the medial PFC and the anterior cingulate (ACC) cortices (Etkin, et al., 2011), b) top-down control by the PFC playing a role in resilience to stress (van der Werff, et al., 2013) and c) chronic stress inducing volumetric atrophy of the PFC *via* the action of glucocorticoids (Cerqueira, et al., 2007; MacLullich, et al., 2006), one would expect better PFC-associated neurocognitive function along with better PFC structural outcomes associated with resilience. More specifically, better PFC- associated neurocognitive and structural outcomes may be attributed to specific hemispheres, based on the suggested lateralized disturbances of brain structure or function, most notably in the PFC, which have been reported in patients suffering from major depressive and anxiety disorders (Davidson, 1998; Johnstone, et al., 2007)   In general, the left hemisphere has been shown to be more involved in verbal processing while the right is more involved with spatial processing (Hellige, 1993) and other generalized distinctions regarding specializations attributed to the left *versus* the right hemispheres include local *versus* global attention (Weissman & Woldorff, 2005) categorical *versus* coordinate spatial representations (Kosslyn, et al., 1989), positive *versus* negative emotions (Hellige, 1993) and encoding *versus* retrieval associated with episodic memory (Cabeza, et al., 2003).

In the context of stress, specifically, evidence has pointed towards the left brain inhibiting right-side-dependent stress-related emotional expressions *via* inter-hemispheric inhibition (Denenberg, 1983; Sullivan, 2004). Furthermore, emotional stimuli presented selectively to the right hemisphere have been shown to remarkably raise cortisol levels, denoting an acute stress response (Wittling, 1997). These findings suggest that better neurocognitive function associated with the left PFC, would render an individual more resilient in the face of stress as this may denote an increased capacity for the left PFC to inhibit deleterious functions associated with

the right hemisphere. Indeed, our data provided support for this notion seeing that resilience (better coping), as assessed by the CD-RISC, was associated with better left PFC neurocognitive function and higher levels of self-esteem was associated with larger left PFC volume. Similarly, evidence exists for specifically left PFC involvement in strategic emotional intelligence (Leopold, et al., 2012), likely to be implicated in coping and high self-esteem. However, our study is the first to investigate neurocognitive function in addition to volumetric differences between the left and right hemispheres, in the context of resilience and self-esteem.

**5.2.4.2 Prefrontal processing dictates physiological outcome**

In general, in addition to the PFC playing an important role in the integration of cognitive and affective behaviour, it also regulates autonomic, neuro-endocrine and –immune functions. The PFC modulates the response to stress through regulation of the hypothalamic paraventricular nucleus (PVN) which, in turn, controls sympatho-adrenal and hypothalamic–pituitary–adrenal (HPA) activity, with the latter serving as the link between the central nervous system and the immune system (Cerqueira, et al., 2008). We therefore now turn to a discussion of our findings pertaining to neurocognitive and structural associations between the PFC and the immune system. Improved neurocognitive function allocated to the PFC was consistently associated with both higher leukocyte counts and higher GR expression. As discussed in section 5.2.2, decreased peripheral leukocyte counts in combination with increased GR expression may reflect a compromised immune system. However, in the case of increased leukocyte counts along with increased GR expression in these different cell types, this may signify a favorable condition. This is based on the notion of more immune cells in circulation for surveillance purposes, along with these cells being more sensitive to regulation by cortisol resulting in decreased inflammation. Conversely, a negative association between PFC cognitive function and neutrophil counts as well as monocyte GR expression was evident. Monocytes and neutrophils are involved in innate immune system-associated inflammation, suggesting that poorer PFC control may be associated with a pro-inflammatory state. In line with this, one other study provided evidence for peripheral activation of the innate immune system associated with impaired working memory (Sparkman, et al., 2006).

The fact that specifically better left PFC hemisphere functioning was consistently associated with increased GR expression further provided evidence for a favorable immune profile in terms of higher GR expression, which might have been a function of increased left PFC inhibition of anxiety-induced amygdala activity, as discussed above. This proposed favorable outcome associated with the left PFC was also mirrored in the observed increased DHEAs levels associated with the left PFC. However, GR expression was not associated with any

brain region in terms of structural volume. This may suggest that (mal)adaptation at the level of GR expression had not taken place over a considerable amount of time, i.e. years, for long term central correlates to be associated with the increased GR expression, as was observed in the current study. This was expected, given that fluctuations in GR expression is more stable than, for example, hormone levels, but up- or down-regulation of the GR is still dependent on cortisol dynamics which fluctuate according to acute stress levels and metabolic demands (Sapolsky, et al., 2000). Accordingly, in the current study's population, adaptation of the GR as a function of hypo-activation of the HPA-axis, might have only have set in relatively recently.

The fact that an inverse relationship was evident between lymphocyte counts and the right PFC, which was in contrast with the positive association between T cell count and right amygdala volumes, may reflect a scenario of PFC-associated inhibition of the amygdala and subsequent immune activation, associated with a loss of lymphocytes from circulation. Assessing the right *versus* the left thalamus when considering associations with physiological parameters did not prove to be useful, seeing that a given marker would correlate with both the right and the left thalamus. This may have indicated a lesser degree of lateralization in this particular brain region, when compared to the PFC, amygdala and hippocampus. However, larger thalamus volumes seemed to signify a more favorable outcome since it was associated with increased levels of DHEAs. Finally, as observed with associations with other parameters, DHEAs, and not cortisol, showed significant correlations, in this case with better neurocognitive function. At this point in time, no literature on neurocognitive function or thalamus grey matter volume in relation to DHEAs and cortisol is available for comparison.

### 5.2.5 Experimentally induced psychosocial stress upon HPA-axis suppression

We have selected the 1mg betamethasone dose based on the fact that this dose had been accepted as the standardized dose for HPA-axis suppression, in adolescents (Dahl, et al., 1992; Duval, et al., 2004). In general, the 1 mg dexamethasone suppression test (DST) had been found to demonstrate cortisol non-suppression (post-dexamethasone cortisol level >5 µg/dl (138 nmol/L)) in approximately 40%–60% of depressed subjects, where the 1mg DST has been optimized for the detection of decreased HPA axis feedback sensitivity (Grossman, et al., 2003). However, PTSD has been associated with DST findings that are essentially the opposite of those in major depression with increased cortisol suppression post Dex injection (Grossman, et al., 2003). Although the 1mg dose has been accepted as the standard dose in adolescents, (Dahl, et al., 1992), studies in children/adolescents have also used the lower doses (0.25mg or 0.5mg) (Birmaher, et al., 1992; Grossman, et al., 2003; Lipschitz, et al., 2003). Furthermore, controversies exist regarding the selection of dosages for children, calculated according

to body surface area (Hindmarsh & Brook, 1985) where Naylor *et al* (Naylor, et al., 1990) promoted such an approach, whereas Doherty *et al* reported no significant difference in the percentage of psychiatrically hospitalized children who failed to suppress at high, medium or low dose of Dex per kg body weight, compared to when a 1mg of standard dose was used in all of the 97 children assessed (Doherty, et al., 1986). Taken together, given that a) the 1mg dosage has been accepted as the standard dose in specifically adolescents, b) the lack of evidence supporting the use of dosages calculated according to body surface area and c) that lower dosages have been used mostly in the context of clinically diagnosed PTSD where these individuals present with significant hypo-activity of the lower HPA-axis (see Chapter 2), we have chosen the 1 mg dose for our "apparently healthy" population. Moreover, because our study population did not present with clinical anxiety (e.g. PTSD) and thus presumably did not have severely low HPA-axis reactivity as is the case with PTSD, we rejected the use of the 0.25- or 0.5- mg dose. Logistically, it was not possible to evaluate HPA responsiveness prior to commencement of the suppression and TSST procedures. In retrospect, given indication of HPA-axis hypo-responsivity in our population, a lower betamethasone dosage may have been a better choice.

Both pre- and post TSST ACTH levels were non-detectable for the majority of participants. Furthermore, cortisol levels were also significantly suppressed, ranging from 11 to 37 nmol/L pre-TSST, compared to studies using a lower Dex dosage in adults (0.5mg), who reported average early morning cortisol levels of a) 15.2 µmol/L (42 nmol/L) for traumatized and 22 µmol/L (60 nmol/L) for healthy controls (De Kloet, et al., 2007) , b) 46 nmol/L for traumatized and 89 nmol/L for healthy controls (Stein, et al., 1997), and c) for traumatized 4.2 µg/dL (11 nmol/L) and 4.6 µg/dL (13 nmol/L) for healthy controls (Newport, et al., 2004). This raised concern in that unlike what we anticipated, our population already presented with significant HPA-axis hypo-activation/reactivity, as was reflected with the enhanced Betamethasone suppression. This may also suggest that the increased peripheral GR expression observed in high anxiety prone individuals might have reflected this enhanced central negative feedback that was evident in our population. Furthermore, given the fact that enhanced Betamethasone suppression was observed even in the case of individuals not being exposed to high levels of trauma and not exhibiting high levels of anxiety proneness, suggested that our particular study population as a whole presented with relative hypoactivity of the lower HPA-axis, denoting risk associated with this population's socio-demographic background and potential stable maladaptation (e.g. at epigenetic level). Future research by our group will involve the investigation of such possible epigenetic effects. We suggest that future studies on similar socio-demographic populations take this into consideration and perhaps use a lower Dex/betamethasone dose. A further suggestion involves comparison of the pharmacological actions of Dex

*versus* Betamethasone as there is some evidence of slight differential biological actions (Dunn, et al., 2010; Zia, et al., 2015).

Despite the relatively severe magnitude of HPA-axis suppression in our study, some interesting results have been obtained. For example, both trauma and anxiety proneness had a diminishing effect on state anxiety, which was measured immediately before the TSST; with paradoxically lower levels of state anxiety associated with higher trauma levels. This might be due to the fact that high anxious, high trauma exposed individuals experienced frequent bouts of state anxiety, due to their anxious dispositions and in response to encountering maltreatment on a frequent basis. Therefore, the type and severity of trauma they have inevitably become desensitized to, were much greater than the mere anticipation of them participating in an event as part of a research project. In contrast, for the individuals who were rarely exposed to any form of complex trauma, a visit to the University Medical School and the knowledge that it will involve blood draws, may have posed a relatively big psychological stressor. Indeed, trait anxiety has been found to reflect individual differences in the sensitivity to threat (Endler & Kocovski, 2001; Eysenck, 1997; Spielberger, 1983). Accordingly, high trait anxious individuals have a tendency to generalize stress-induced bias to an imminent stressor, by inflating the subjective risk of negative events unrelated to this current stressor (Butler & Mathews, 1987). Therefore, in the context of the current study, when state anxiety was elevated by an impending danger, low trait anxious individuals perceived increased risk to be associated with the particular danger, while high trait anxious individuals perceived increased risk everywhere.

This phenomenon was also supported by the fact that as expected, perceive state anxiety (as measured by a visual analogue scale) increased significant after the TSST in association with anxiety proneness, with this increase more pronounced in the low anxiety groups. This finding may imply that state anxiety, as measured by the STAI and a VAS, is context specific, and caution should therefore be taken in using these scales other that investigating reactivity to a challenge, i.e., in basal states. Furthermore, higher levels of perceived anxiety scores, both before and after the TSST, were correlated with a higher degree of consistent right-handedness. This is in line with another study showing that with adult right handers, a higher degree of consistent right-handedness was associated with greater anxiety than inconsistency (mixed handedness) (Lyle, et al., 2013). These findings, together with the tendency in the current study for a higher degree of consistent right handedness to be associated with poorer right hemisphere-associated visuospatial memory, provide support for

Annette's right-shift theory (see Chapter 2 section 2.4.4.2). This theory posits that that mixed handed individuals present with an evolutionary advantage to their pure left and right handed counterparts.

**5.2.6 Summary: Identifying markers of anxiety proneness - contributing to a precision medicine paradigm**

Considering section 5.2, we concluded that in relatively low socio-demographic populations such as in the current study, a) over time, serum levels of DHEAs can be used as a marker in monitoring the efficacy of anxiety prone-focused treatment/therapy, as well as the efficacy of therapy aimed at improving neurocognitive function associated with the PFC; b) GR expression assessment can be used in the monitoring of anxiety prone-focused treatment outcome; c) in terms of identifying individuals who would benefit from treatment with anti-inflammatory medicine, serum levels of IL-12p70 can be used to identify the presence of systemic inflammation in anxiety-prone individuals as well as in individuals subjected to, specifically, emotional and physical abuse (although more research is required to validate this marker); d) with therapeutic interventions targeting deficits in neurocognitive function pertaining to the PFC, outcomes on the SSAIS and the TOL can be tracked over time, so as to assess therapeutic progress and/or efficacy in high- anxiety-prone and -trauma exposed individuals; d) levels of state anxiety are advised to be used in the assessment of effects associated with anxious arousal and not anxious apprehension, as this marker is largely dependent on the immediate context; and e) the EHI is an an-easy-to-measure indicator of risk/resilience in the perception of level of anxiety, depending on the degree of consistency (in right handers).

**5.3 Potential outcome modulating factors in the context of anxiety proneness and trauma**

**5.3.1 Handedness**

Since handedness is a stable characteristic, established very early in life, it was not surprising that trauma had no significant effect on handedness. This is supported by evidence for signs of handedness observed as early as before birth (McCartney & Hepper, 1999). However, infants initially use both hands indifferently (Corbetta & Thelen, 1999; Rönnqvist & Domellof, 2006) and preference for one hand only becomes clear at around 18 months of age (Fagard & Marks, 2000), with this tendency increasing during the following years (Dubois, et al., 2009). Right-handedness has been associated with lateralization of language during the first 2 years of life (Nelson, et al., 2014). Furthermore, the CTQ measures self-reported childhood trauma prior to the age of 18. However, most individuals cannot accurately account for traumatic events which have occurred during the first

year of life, i.e. during the time when handedness is established. Thus, in our opinion, a CTQ score can therefore not provide an indication of environmental factors influencing the establishment of handedness during the above indicated critical period.  This, together with the facts that a) more or less 90% of the general non-clinical population is right handed (Laland, et al., 1995) and b) once handedness is established, it is considered an enduring trait (Michel, et al., 2014), despite it not being entirely hereditable (see Chapter 2, section 2.4.4.2), it is not surprising that we had only right handed participants in our study and there was no effect of trauma on the varying degrees of consistent right-handedness observed.

In terms of anxiety proneness, our study did not support a notion of differences in hemisphere lateralization between different degrees of right handers, which may be associated with a predisposition towards anxiety proneness.   This may have suggested that variations in lateralization of neurocognitive functions associated with anxiety proneness, within the relatively small bracket of right-handedness (as opposed to variations between right *versus* left handedness), were not significant. This idea is supported in the literature where even between the larger extremes of left and right handed individuals, only a slight difference in brain asymmetry exists, albeit in terms of language functions (Corballis, 2014). Furthermore, a multi-modal construct such as anxiety proneness is dependent on many neurological functions, dispersed throughout the brain (left and right hemispheres), and therefore cannot be generalized to be comprised of functions lateralized to either the left or right hemisphere. Therefore, if lateralization of a lesser order, single factor associated with anxiety proneness, such as emotional lateralization, has to be assessed in terms of handedness, an effect of handedness might be observed. Also, an effect of handedness might be more pronounced when an assessment is made between measures associated with anxiety proneness and pure left-handed individuals, in comparison with pure right-handed and mixed-handed individuals. However, an investigation of these suggestions is beyond the scope of the current thesis.

**5.3.2. Resilience**

Although we did not report results for the initial cohort of 1149 participants from which the current study's 43 participants were selected - this larger cohort was used for the main purpose of creating upper- and lower-percentiles in the delineation into the four experimental groups - it is noteworthy to consider that additional statistics from the 1149 participants yielded significantly negative correlations of resilience with both anxiety proneness (r = 0.174) as well as trauma (CTQ total) (r = 0.307), and these correlations were mirrored when trauma and anxiety proneness were correlated with coping, as measured by the ACOPE.  Interestingly, in this

larger cohort, the number of negative life events – life events are considered as extreme traumatic events- was positively correlated with resilience ($r = 0.131$) and when the subscales of the CTQ were considered in the analysis, all scales correlated negatively with resilience except for emotional- and sexual- abuse, which showed no correlation. Our interpretation of these additional results, with high statistical power, is that extreme traumatic life events may instill the belief that "If I can endure such severe mishaps, I am capable of dealing with anything that life presents". The more exposure an individual then has to these extreme events, the more resilience (or at least the perception of being more resilient) the individual displays, thus being more able to cope with life's demands. In contrast, with childhood maltreatment, which is of a lesser intensity than traumatic life events, but occur more frequently over time, resilience would be less. This is based on the supposition that in the case of constant environmental discord, there is very little "room left for coping", as there is a constant demand for the individual's psychobiological systems to react to these forms of maltreatment. Therefore, when interpreting data associated with the CD-RISC, it is important to also investigate the specific types as well as the intensity of the trauma individuals were exposed to.

Furthermore, scores on resilience using the CD-RISC need to be interpreted in light of the specific population under investigation. To illustrate just how diverse scores of populations considered "normally healthy" are, we provide two mean scores obtained from the Unite States of America, for healthy controls and federally recognized elderly Native Americans ($82.7 \pm 8.0$ and $83.0 \pm 13.4$ respectively) (Goins, et al., 2012; Sutherland, et al., 2009) *versus* a mean score of healthy young adult Korean volunteers ($66.8 \pm 12.7$)(Ha, et al., 2009). Our study's mean score for the low anxiety proneness, low trauma group ($73.4 \pm 13.8$), lied somewhere in between the USA and Korean scores. However, it was higher than 3 representative samples of high school children in South Africa reported a few years ago ($64.8 \pm 18.9$; $65.9 \pm 18.6$ and $63.7 \pm 17.9$) (Bruwer, et al., 2008; Fyncham, et al., 2009; Jorgensen & Seedat, 2008), as well as higher than the scores of adolescent refugees from Africa, Yugoslavia and the Middle East who were living in South Australia, with respective mean scores of 60, 69, and 67 (Ziaian, et al., 2012). However, in stark contrast, in the current study population, for the group with both high trauma and high anxiety proneness, resilience scores ($58.6 \pm 14.6$) were lower than all of these populations. From this, it seems probable that either high anxiety proneness, or high trauma, or both, decreased resilience in the group studied. However, no significant main effect of anxiety proneness on resilience was observed in the current study. Possible reasons for this unexpected result are provided in section 5.4.

### 5.3.3. Self-esteem

The positive association between trauma and self-esteem was at first puzzling, as it does not support either the vulnerability (van Tuijla, et al., 2014) or the scar models (Harter, 1999) of self-esteem. Since the R-SES measures levels of global trait self-esteem, it seemed unlikely that this finding was due to the placebo effect of being selected to participate in a study. However the inoculation hypothesis does lend support to this finding – according to this hypothesis, mildly traumatic insults can serve to boost resilience, and self-esteem has been posited as a marker associated with resilience against stress (Zeigler-Hill, 2010). We are of the opinion that the association we report is specific to our study population, and not generally applicable. A further discussion of this unusual finding is provided in section 5.4.

### 5.3.4 Potential outcome modulating factors in practice – support from current data

We propose that all three questionnaires (i.e. CD-RISC, EHI, and R-SES) can be used in the identification of neurophysiological markers of either resilience (correlated with high scores on the CD-RISC and R-SES and a high degree of consistent right handedness) or vulnerability (correlated with opposite outcomes on these questionnaires). We will therefore discuss correlations with these potential OMFs, on the backdrop of relationships with anxiety proneness and trauma.

In terms of the EHI, a higher degree of consistent right handedness was associated with lower total WBC-, neutrophil- and T cell counts. More support for a higher degree of consistent handedness (in our case, right handedness) being associated with more risk/vulnerably stems from the fact that more consistent hand preference has been associated with smaller corpus callosum size (e.g., (Luders, et al., 2010)) and decreased inter-hemispheric interaction and with decreased right hemisphere access as well as increased likeliness to ruminate (Niebauer, et al., 2004), while higher degrees of inconsistent (mixed) handedness have been associated with superior episodic memory retrieval and increased belief updating/cognitive flexibility (Prichard, et al., 2012), regardless of the direction (left or right) of consistent hand preference (Lyle, et al., 2012). In terms of the function of belief updating/cognitive flexibility, when something challenges pre-existing beliefs, inter-hemispheric interaction serves the purpose of conveying the inconsistencies detected by the right hemisphere, to the left hemisphere.

In terms of the CD-RISC, resilience correlated with increased GR expression, which in turn, has been associated with anxiety proneness. This needs to be interpreted in light of the CD-RISC scale measuring current resilience,

and not providing an indication of resilience as a global trait function. Accordingly, a measure of trait resilience can be more accurately compared to the probable chronically maladapted levels of GR expression. Furthermore, in the case of an individual "deemed as resilient", resilience in this sense is a multimodal faculty dependent on a myriad of different functions. Therefore, in our opinion, a) the CD-RISC cannot be used in association with markers indicating adaptation which has taken place over a matter of months or years and b) increased expression of GR associated with anxiety proneness may be an indication of vulnerability and not resilience. Accordingly, a recent article has provided evidence for high GR expression level in PBMCs to be associated with increased risk for developing PTSD (van Zuiden, et al., 2013).

When studying resilience with regard to HPA-axis activity, the context of the stressor such as ongoing family violence as well as the availability of internal and external coping resources must be considered (Hostinar, et al., 2013). Accordingly, resilience is thought to be malleable and responsive to the social environment (Herrenkohl, 2013; Klika & Herrenkohl, 2013; Ungar, 2013), where different types of trauma may effect resilience in different ways. In our population, resilience was negatively correlated with trauma, in particular, physical neglect and abuse. However, given our relatively small sample size of 43 participants, drawing conclusions in terms of the different subscales of trauma may be problematic. In terms of the literature, to the best of our knowledge, only one study has explored the relationship between resilience and trauma subscales:  in young adults, resilience was shown to moderate the relationship between emotional neglect and current psychiatric symptoms (Campbell-Sillsa, et al., 2006). We therefore highlight the necessity for more studies to be conducted, focusing on better defining resilience and providing evidence for the construct validity of the Connor–Davidson Resilience Scale in terms of a low socio-demographic population.

In terms of self-esteem, measured by the R-SES, trauma, in particular emotional and sexual abuse, was positively correlated with self-esteem. In our opinion, for trauma to increase self-esteem, there is likely to be specific functions of trauma as well as dynamics of the specific study population, at play, which could result in something as detrimental as a history of childhood maltreatment to increase levels of self-esteem. Accordingly, specific to the study of childhood or adolescent sexual abuse, the quality of the family environment (Fassler, et al., 2005) and of the victim's attachment experiences have been described as important moderating, or mediating factors on psychosocial and psychopathological consequences of sexual abuse (Barker-Collo & Read, 2003; Shapiro & Levendovsky, 1999). Given the interrelatedness of self-esteem with attachment schemas, we suggest that future studies include a measure of attachment style in research assessing self-esteem as a marker of

resilience, so as to shed more light on possible variables at play. A discussion of possible confounding matters in the assessment of self-esteem follows in more detail, in the next section.

**5.4 Population characteristics and/or conditions to consider when interpreting data**

Some unusual results were obtained from our investigation of this population. Although not directly assessed in this adolescent population – some because of the very sensitive nature of such investigations - we could speculate on reasons for at least some of the unexpected results.

In our population, anxiety proneness had no effect on resilience, in contrast, trauma seemed to decrease resilience, albeit only a tendency. The lack of association between resilience and anxiety proneness was somewhat unexpected. Therefore, in the following paragraphs, we discuss possible confounding factors which may skew resilience outcome, as assessed by the CD-RISC.

In our opinion, interpretation of the items on the CD-RISC may have been fairly subjective and culture-dependent. For example, item 18 states "In dealing with life's problems, sometimes you have to act on a hunch, without knowing why" and item 20 states "I can make unpopular or difficult decisions that affect other people, if it is necessary". Indeed, there exists strong evidence pointing towards the idea that friends, family, spirituality, schools and other community groups can inculcate resilience (Marriott, et al., 2014), and therefore and idea of resilience or lack thereof may be instilled in an individual by the social environment, and this idea might not necessarily reflect true resilience. Furthermore, the CD-RISC is comprised of items formulated according to Western literature and concepts (Ungar & Liebenberg, 2011), and we therefore question the validity of this scale in terms of use in research across all societies of the world. In the same vein, a measure of resilience could be influenced by variables such as social support and community factors (Herrenkohl, 2013; Marriott, et al., 2014), where in this case, a resilience outcome measure would not be a function of an individual's coping capacity *per se*, but rather of external social influences.

In light of the fact that there was no effect of anxiety proneness on resilience and there was only a tendency for trauma to decrease resilience scores, we postulated the following:

a. Resilience is a pre-existing hereditary factor and is stable over time, i.e., not influenced by anxiety or trauma. However, the CD-RISC measures resilience pertaining to perceived level of coping in the last month, and therefore resilience measured by means of the CD-RISC, is not an enduring, hereditary trait.

b. The number of participants assessed in the current study was too small to provide statistical power for more statistically significant relationships to be revealed. However, this is unlikely given the many other results obtained on more variable parameters, such as leukocyte counts and DHEAs levels.

c. Measures on the CD-RISC did not accurately reflect resilience in our population, due to culture-specific effects.

d. Resilience is differently affected by the type and intensity of trauma, so that what we have measured statistically using the CTQ excluded the more extreme life events that could potentially influence resilience.

From this we conclude that resilience is a product of a combination of factors, where scores on the CD-RISC should be viewed in the context of the above outlined confounding factors. Once more clarity has been gained regarding the use of the CD-RISC and the scale has been accordingly adapted for low socio-demographic populations, it can be used to identify specific markers of resilience in terms of neurophysiology.

Turning to self-esteem, at first, the seemingly contradictory effects of trauma on self-esteem seemed puzzling, with trauma seemingly exhibiting a positive effect on self-esteem. However, we proposed a number of possible explanations for the positive effect of trauma on self-esteem. Firstly, most of the participants in the current study were living under low socio-economic conditions, in townships where children and parents often share the same bedroom. This scenario could create a breeding ground optimal for domestic violence and sexual abuse to take place, simply due to increased proximity between family members. However, in these cultures, these circumstances may be considered the norm and these children may be desensitized to maltreatment, and/or they may interpret these behaviors of caretakers to be a type of "attention-giving conduct", albeit in the form of abuse or violence.

Considering the current study population, a second possible explanation involves the belief that survival of harsh conditions results in being stronger and/or streetwise than before. However, taking on this stance, without cognitive integration of traumatic events, may signify a defense mechanism denoting self-competence at the expense of self-liking. If this is the case, the unintegrated unconscious effects of trauma may still run their course in terms of neurophysiological maladaptations while simultaneously exhibiting high self-esteem, as measured by the R-SES. This may be due to the fact that self-esteem is based on both the positive or negative reflection that the individual has for himself/herself as well as the belief of being able to cope with the basic challenges of life (Galanou, et al., 2014). Indeed, some scholars argue that the internal structure of the

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Rosenberg scale is assumed to be mono-dimensional, however, assessment of the R-SES showed the existence of two facets of self-esteem: a) denominated self-competence (items 3, 4, 5, 7, 9) and b) self-liking (items 1, 2, 6, 8, 10) (R-SES) (e.g. (Greenberger, et al., 2003; Tafarodi & Milne, 2002)). Therefore, self-efficacy has been generalized to self-esteem, due to, in particular , items 3, 4, 5, 7, 9, which represent self-competence evaluation constructs closely related to Bandura's (Bandura, 1977) concept of general self-efficacy (Roth, et al., 2008). In the absence of satisfying relationships, along with being fearful - as is the case with insecure attachment which may be a potential core feature of our study population - an individual might obtain satisfaction through other means, thus maintaining a high level of self-esteem (e.g., (Huntsinger & Luecken, 2004) Also, the wording of the R-SES may be such that it is interpreted differently in different cultures, as was indeed evident in the administration of the R-SES in 53 nations, yielding variable data in terms of the self-competence and self-liking subscales with cultural individualism (Schmitt & Allik, 2005).

Furthermore, as discussed in Chapter 2, section 2.4.4.3, self-esteem is based on both I-self and Me-self concepts. However, it has been proposed that childhood maltreatment can lead to disturbances in especially the Me-self (Harter, 1999), which is categorical in that it creates categories by which to define the self. Therefore it is possible that childhood maltreatment could lead to the construction of a 'theory' so as to organize one's thinking about one's relationship to the social world; one which could be positive even with (or because of) the experience of a history of childhood maltreatment.

Interestingly, both the CD-RISC and R-SES which require to be interpreted in light of the specific study population, were influenced by trauma and not anxiety proneness. This provided more evidence for the notion that these two scales are highly dependent on environmental factors, more so than inherent characteristics.

Finally, on a physiological level, our study population as a whole was unique in the sense that the participants' immune systems seemed under-activated. This was based in part on the overall low-normal WBC count (indicating absence of pathological suppression), but most significantly the abnormally low cytokine profile across the group. The latter finding may suggest that in this population, similar to the suggested hypoactivtion of the lower HPA-axis (as indicated by the below normal levels of DHEAs for the whole group), there is also a general blunting of the cytokine signaling system. The mechanism for this maladaptation remains to be elucidated. One potential explanation is that the probable immune suppression observed may have been a result of transgenerational transfer of epigenetic modulation in the context of HPA-axis and/or cytokine-related genes in response to the specific socio-demographic setting of our population (see section 5.1). This (socio-

demographic environment) could entail relatively poor nutritional status as it is well established that protein-energy malnutrition, especially, is associated with a significant impairment of cell-mediated immunity, phagocyte function, complement system, secretory immunoglobulin A antibody concentrations as well as cytokine production (Chandra, 2002).  Another possible explanation for the low cytokine profile could be parasite infection, as a study on multiple helminth infections and parasite burden in a large cohort revealed geometric mean values for IL-1β, IL-5, IL-6, IL-10, and for IL13 that were low and close to the respective detection limits of each assay, considering less than 30% of participants as responders (Geiger, et al., 2013). However, the latter seems improbably, given the fact that white cell counts did not suggest parasitic infection.

**CHAPTER 6**

**CONCLUSIONS**

In conclusion, in terms of practical management of populations at risk for development of chronic illnesses/conditions as well as psychopathology, we have formulated the following guidelines:

1.  Levels of DHEAs and not cortisol should be used in monitoring levels of anxiety proneness, especially when neurocognitive and structural outcomes are targeted by means of intervention.

2.  GR expression level is a relatively efficient marker easily obtained from a peripheral blood sample, and is more stable across time, when compared to leukocyte counts which fluctuate with disease. However, since, up to date, GR expression has not been researched in the context of anxiety proneness and trauma, suitable reference ranges have not been established. Furthermore, due to the limitation that a more recent MFI standardization technique was not employed in the current study, our results should be further validated. Therefore, possible future research may involve the validation of our findings as well as the determination of ranges specific to anxiety *versus* that of trauma exposure in the South African low socio-economic context. These could be used to monitor patients over time.

3.  Given the observed compromised PFC deficits in information processing and signs of amygdala hyperactivity observed in specifically anxiety proneness, we suggest that therapy in individuals scoring high on anxiety proneness should be focused specifically on improving PFC cognitive control of emotional information processing. Furthermore, outcomes on the SSAIS and TOL can be used to track improvement in neurocognitive function associated with therapy, over time.

4.  Handedness can be used as a measure to identify risk seeing that more consistently right handed individuals may be at greater risk for physiological maladaptation.

Areas of remaining uncertainty that should be addressed include:

1.  In terms of resilience, we propose that a culture-specific scale be constructed for use in the South African context.

2.  We propose that future studies on self-esteem distinguish between the effects of self-esteem which are due to self-liking and those that are as a consequence of perceived self-competence.

3.  That larger population samples be used in the investigation of the effects of specific subtypes of trauma on markers of resilience/risk, so as to tailor-make intervention accordingly.

4. We recommend that physiological maladaptation in at-risk populations be investigated in terms of epigenetics.

Taking the findings of the current study into consideration, we proposed a holistic approach to intervention. Fig. 5.2 below indicates (by means of red suns) the possible areas to target, within a precision medicine framework:

**Figure 5.2** Possible avenues for intervention
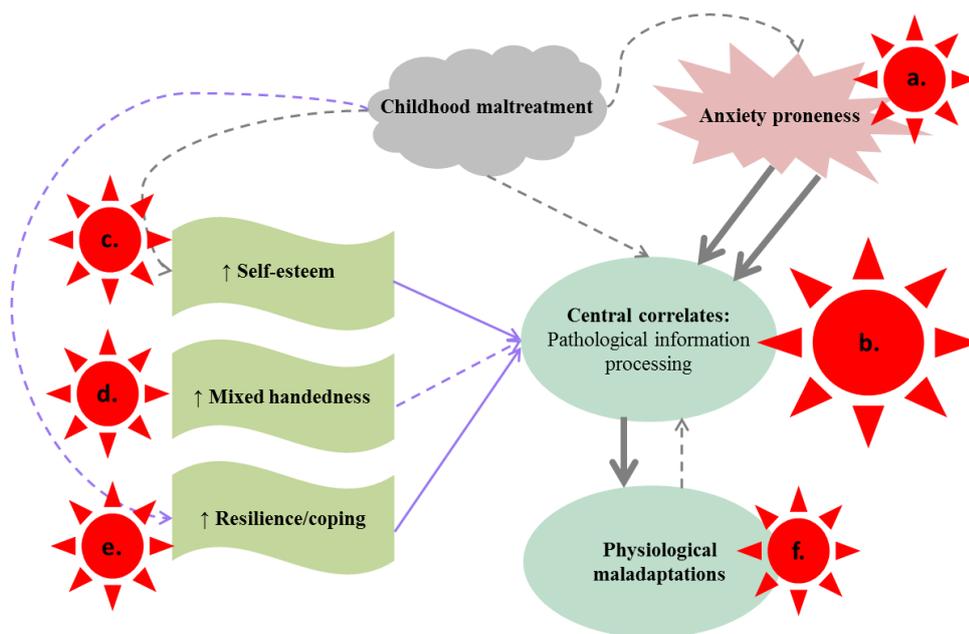


Considering fig 5.2 above, the first avenue to intervene at is at (a.), where at risk populations in terms of anxiety proneness (trauma exposure can be used as an additional marker of risk) can be diagnosed by considering appropriate reference ranges of scores for the STAI-T, CASI, and CTQ (including most relevant trauma subtypes). We suggest that the period of adolescence be considered in this identification of risk, seeing that the onset of anxiety disorders and neurophysiological maladaptations are associated with this age-group and seeing that adolescence present with the developed capacity to do introspection and provide accurate reports of emotions, a capacity which may lack in childhood. At (b.), possibly the best area to intervene, in anxiety prone individuals, baseline (i.e. before intervention) levels of neurocognitive deficits can be assessed to determine the focus of therapy, with follow-ups of these measures used for monitoring purposes over time. Potential therapeutic approaches can involve training in mindfulness (meditation) so as to address mood congruent aspects of information processing (specifically addressing the model of controllability and anxiety, introduced in Chapter 2), as well as improving executive function and other aspects associated with PFC control of emotional information processing, by means of, for example, cognitive behavioral therapy (CBT).

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At the same time, context-specific information processing can be addressed by therapy focusing on increasing self-esteem – by for example, improving self-liking and self-competence aspects of the self, implementing secure attachment to significant others, and creating a sense of belonging - as well as increasing markers that have been positively associated with a measure of culture-appropriate resilience, seeing that self-esteem and resilience have both been found to be uniquely affected by trauma exposure (contextual factors). Furthermore, intervention can also focus on increasing the use of both hands in doing tasks, with the aim of promoting inter-hemispheric interaction/integration). Finally, physiological parameters associated with maladaptation can be tracked over time, to determine the degree of efficacy and/or progress in terms of therapy. This avenue can also be explored in the context of symptomatically treating individuals with either conventional or alternative medicine, which have, for example, anti-inflammatory properties.

However, before these intervention strategies can be implemented, further research is required to determine age-and-population-specific reference ranges in psychometric and neurophysiological outcomes associated with vulnerability, highlighted in the current thesis. This approach in research will contribute to the development of individualized treatment, or 'precision medicine' (Cuthbert & Insel, 2013). Once these appropriate reference ranges have been established, at risk populations can be identified and therapy can center on improving pathological information processing. The focus of therapy can be to provide these individuals with a) tools whereby their beliefs and perceptions about themselves in relation to the world can be challenged, by means of increased self-awareness and executive monitoring (allocentric and existential mode of information processing), even in in the face of traumatic incidences, b) more effective coping strategies which can replace the former pathological cognitive, emotional and behavioural avoidance strategies, and c) the opportunity for extinction of conditioned fear to take place.  Re-framing of ambiguous and/or negative information as, for example, temporary, benign and/or meaningful, addresses pathological information processing rooted in both nature and nurture. This is based on the notion that biology, even on the level of epigenetics, follows belief, which, in turn, is shaped by experience (for a schematic representation, see fig. 2, Chapter 2).

In conclusion, the current study provides strong evidence for the brain-body link. Bearing in mind this interdependence between "mind and matter", in the context of anxiety and childhood maltreatment, is important in the holistic treatment of individuals at risk for psychopathology and concomitant maladapatations of neurophysiological systems. More specifically, we have highlighted markers of resilience and vulnerability which can be used as tools for monitoring and/or managing at risk populations over time, by means of therapy,

and/or conventional or alternative medicine. In our study population of relatively poor socio-demographic backgrounds, anxiety proneness, in general, had a superior effect on neurophysiological outcome, compared to childhood maltreatment. We therefore suggest that future studies on childhood maltreatment include a measure of anxiety and in terms of intervention, anxiety proneness should be the main focus.

**"As the mind, so the person; bandha or moksha (bondage or liberation) are in your own mind." If you feel bound, you are bound. If you feel liberated, you are liberated. Things outside neither bind nor liberate you; only your attitude toward them does that."**

— Swami Satchidananda, The Yoga Sutras of Patanjali: Commentary on the Raja Yoga Sutras by Sri Swami Satchidananda

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**APPENDIX A**

Assent and consent forms

| | | |
|---|---|---|
| ![S logo] | STELLENBOSCH UNIVERSITY<br><br>**FACULTY OF HEALTH SCIENCES** | ![Stellenbosch seal] |
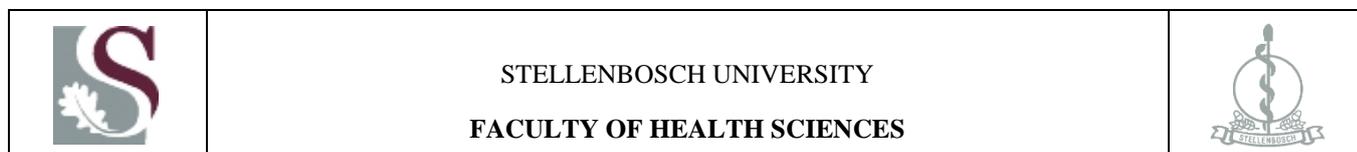
### PARTICIPANT INFORMATION LEAFLET AND ASSENT FORM B



**TITLE OF THE RESEARCH PROJECT:** Correlations between anxiety, childhood trauma, resilience and physiological determinants of health, both centrally and peripherally, in adolescents

**RESEARCHERS NAME(S):** Monet Viljoen

**ADDRESS:**    Department of Psychiatry, Health Sciences Faculty, Stellenbosch University, and Multidisciplinary Stress Biology group, Department of Physiological Sciences, Natural Sciences Faculty, Stellenbosch University

**CONTACT NUMBER:** 021-938 9162; 021-808 4388

**What is this research project all about and why have I been invited?**
You are already participating in another research study. During two of the visits already scheduled, we would like to include some extra tests on you. In this way, we can learn more about how anxiety influences your hormones and your ability to fight off disease-causing agents (we call these pathogens). Being able to combine our data and that of the other study (with Ms Martin) will enable us to make the best possible conclusions.

**Who is doing the research?**
The researcher's name is Monet Viljoen. A nurse and medical doctor will do the blood draws and other tests for her. She will be present all the time, in case you have questions.

**What will happen to me in this study?**
During the first visit for this study, a nurse will draw blood from your arm – 8ml only (less than two teaspoons). We will use this to measure concentrations of hormones in your blood, and we will test your immune function in a tube. You will also be given a Dex tablet to be swallowed on that same evening, which will be prescribed by a medical doctor. This tablet contains a very small amount of synthetic hormone (Dex). The dosage of hormone is so small that it should not affect how you feel.

The next visit will be scheduled for the following morning (the day after taking the Dex tablet) where a nurse will draw blood from your arm again (about four tea spoons). You will also do a speaking and a math task, for which you will not need to study. This test will show us how your body responds to stress.

**Can anything bad happen to me?**
You may feel some pain associated with having blood drawn from a vein.  You may experience discomfort, bruising and/or other bleeding at the site where the needle is inserted.  Occasionally, some people experience fleeting dizziness or feel faint when their blood is drawn. A nurse will be there with you all the time, to make sure that you are OK.

If you feel sick or are in pain as a result of being in the study, please let your parent(s) or legal guardian know.

**Will anyone know I am in the study?**
If you consent to participate in this study, your identity relating to any information you provide us, as well as both your blood samples, will be kept confidential.  All research information and laboratory samples obtained from you will be safely stored and identified by code number so as to maintain and protect your anonymity. Access to your information will be limited to authorised study investigators.

**Who can I talk to about the study?** You can contact Lindi Martin at Tel. 021-9389162 and Monet Viljoen Tel. 021-808 4388; Cell. 073 774 9876 if you have any further queries or encounter any problems.

**What if I do not want to do this?**
It is your choice whether or not you want to take part in this study, even if your parent(s) have agreed to your participation. You can stop being in the study without any problems.

Do you understand this research study and are you willing to take part?

| YES | NO |
|-----|-----|

Has the researcher answered all your questions?

| YES | NO |
|-----|-----|

Do you understand that you can pull out of the study at any time?

| YES | NO |
|-----|-----|


_____          _____
Signature of Child                               Date

| | STELLENBOSCH UNIVERSITY<br><br>FACULTY OF HEALTH SCIENCES | |
|---|---|---|
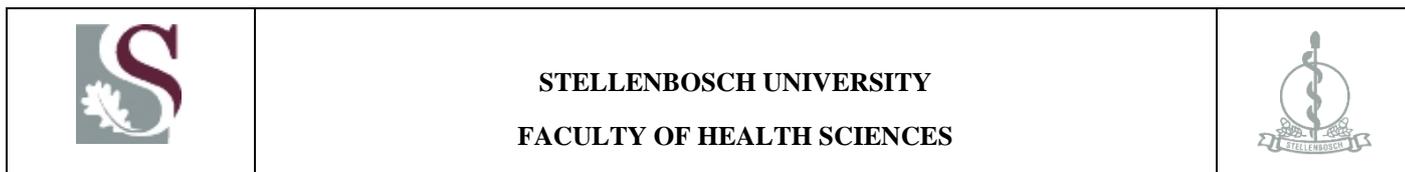
**PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM FOR USE BY PARENT/LEGAL GUARDIANS**

**TITLE OF THE RESEARCH PROJECT**: Correlations between anxiety, childhood trauma, resilience and physiological determinants of health, both centrally and peripherally, in adolescents

**REFERENCE NUMBER:** N11/04/131

**RESEARCHER'S NAME:** Monet Viljoen

**ADDRESS:** Department of Psychiatry, Health Sciences Faculty, Stellenbosch University, and Multidisciplinary Stress Biology group, Department of Physiological Sciences, Natural Sciences Faculty, Stellenbosch University

**CONTACT NUMBER:** Tell: 021-9389162; Cell: 076 774 9876. Please contact Monet with any study-related queries or concerns.

Your child is being invited to take part in a research project. It is important that you are fully satisfied that you understand what this research entails and how your child could be involved. Your child's participation is **entirely voluntary** and you are free to decline to his/her participation. You are also free to withdraw your child from the study at any point, even if you do initially agree to let him/her take part.

**What is this research study all about?**

We are conducting this research to learn more about the relationship between childhood trauma and anxiety proneness in healthy adolescents. We also want to learn more about how the emotional statuses of adolescents are reflected in their immune and endocrine systems. By measuring the levels of specific hormones in the blood and by performing certain experiments in the laboratory, on the cells harvested from the blood, we will be able to see to what degree the immune and endocrine systems are being activated under both conditions of anxiety/stress and conditions of relatively little anxiety. In order for us to learn more about this, two visits are required: a) one will involve your child's blood being drawn by a nurse, and your child taking a Dex tablet which has been prescribed by a medical doctor. The tablet contains only a small amount of synthetic hormone, which resembles a naturally occurring hormone in our bodies. b) the second visit will be scheduled for the following day and will involve another blood draw and a speech and math task. This test will give an indication as to how your child's body responds to stress.

**Declaration by parent/legal guardian**

By signing below, I *(name of parent/legal guardian)* …………………………………...……. agree to allow my child (name of child) …………………………………… who is ………. years old, to take part in the above-named **research** study. **\*I declare that:** (1) I have read or had read to me this information and consent form and that it is written in a language with which I am fluent and comfortable; (2) your child must agree to take part in the study and his/her ASSENT must be recorded on the form provided to him/her; (3) I have had a chance to ask questions and all my questions have been adequately answered; (4)I understand that taking part in this study is **voluntary** and I have not been pressurised to let my child take part; (5) I may choose to withdraw my child from the study at any time and my child will not be penalised or prejudiced in any way; (6) My child may be asked to leave the study before it has finished if the study doctor or researcher feels it is in my child's best interests, or if my child does not follow the study plan as agreed to.

Signed at (place) _____ Date: _____

Signature of parent/legal guardian: _____ Signature of witness: _____

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**APPENDIX B**

The Child Anxiety Sensitivity Index (CASI), the Trait Anxiety version of the State-Trait Inventory (STAI-T),

and the Childhood Trauma Questionnaire (CTQ)

**CASI**

Participant initials: _____        Participant number: _____        Date: _____

A number of statements which boys and girls use to describe themselves are given below. Read each statement carefully and put an 'X' on the line in front of the words that describe you. There are no right or wrong answers. Remember, find the words that best describe you.

|    |                                                                                              | **None** | **Some** | **A lot** |
|----|----------------------------------------------------------------------------------------------|----------|----------|-----------|
| 1  | I don't want other people to know when I feel afraid.                                         | 1        | 2        | 3         |
| 2  | When I cannot keep my mind on my schoolwork, I worry that I might be going crazy.             | 1        | 2        | 3         |
| 3  | It scares me when I feel 'shaky'.                                                             | 1        | 2        | 3         |
| 4  | It scares me when I feel like I am going to faint.                                            | 1        | 2        | 3         |
| 5  | It is important for me to stay in control of my feelings.                                     | 1        | 2        | 3         |
| 6  | It scares me when my heart beats fast.                                                        | 1        | 2        | 3         |
| 7  | It embarrasses me when my stomach growls (makes noises).                                      | 1        | 2        | 3         |
| 8  | It scares me when I feel like I am going to throw up.                                         | 1        | 2        | 3         |
| 9  | When I notice that my heart is beating fast, I worry that there might be something wrong with me. | 1    | 2        | 3         |
| 10 | It scares me when I have trouble getting my breath.                                           | 1        | 2        | 3         |
| 11 | When my stomach hurts, I worry that I might be really sick.                                   | 1        | 2        | 3         |
| 12 | It scares me when I can't keep my mind on my schoolwork.                                      | 1        | 2        | 3         |
| 13 | Other kids can tell when I feel shaky.                                                        | 1        | 2        | 3         |
| 14 | Unusual feelings in my body scare me.                                                         | 1        | 2        | 3         |
| 15 | When I am afraid, I worry that I might be crazy.                                              | 1        | 2        | 3         |
| 16 | It scares me when I feel nervous.                                                             | 1        | 2        | 3         |
| 17 | I don't like to let my feelings show.                                                         | 1        | 2        | 3         |
| 18 | Funny feelings in my body scare me.                                                           | 1        | 2        | 3         |

**STAI-T**

Participant initials: _____      Participant number: _____          Date: _____

**Instructions**: A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate answer 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 disturbing 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 & bothers me. | 1 | 2 | 3 | 4 |
| 18 | I take disappointments so keenly that I can't put them out of my mind. | 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 |

**CTQ-SF**
Copyright 1996 David P. Bernstein, Ph.D., Laura Fink, Ph.D.

Participant initials: _____     Participant number: _____     Date: _____

**Instructions:** These questions ask about some of your experiences growing up **as a child and a teenager**. For each question, circle the number that best describes how you feel. Although some of these questions are of a personal nature, please try to answer as honestly as you can. Your answers will be kept confidential.

| When I was growing up … | Never True | Rarely True | Sometimes True | Often True | Very Often |
|---|---|---|---|---|---|
| 1. I didn't have enough to eat. | 1 | 2 | 3 | 4 | 5 |
| 2. I knew there was someone to take care of me and protect me. | 1 | 2 | 3 | 4 | 5 |
| 3. People in my family called me things like "stupid", "lazy", or "ugly". | 1 | 2 | 3 | 4 | 5 |
| 4. My parents were too drunk or high to take care of me. | 1 | 2 | 3 | 4 | 5 |
| 5. There was someone in my family who helped me feel important or special. | 1 | 2 | 3 | 4 | 5 |
| 6. I had to wear dirty clothes. | 1 | 2 | 3 | 4 | 5 |
| 7. I felt loved. | 1 | 2 | 3 | 4 | 5 |
| 8. I thought that my parents wished I had never been born. | 1 | 2 | 3 | 4 | 5 |
| 9. I got hit so hard by someone in my family that I had to see a doctor or go to the hospital. | 1 | 2 | 3 | 4 | 5 |
| 10. There was nothing I wanted to change about my family. | 1 | 2 | 3 | 4 | 5 |
| 11. People in my family hit me so hard that it left bruises or marks. | 1 | 2 | 3 | 4 | 5 |
| 12. I was punished with a belt, a board, a cord, or some hard object. | 1 | 2 | 3 | 4 | 5 |
| 13. People in my family looked out for each other. | 1 | 2 | 3 | 4 | 5 |
| 14. People in my family said hurtful or insulting things to me. | 1 | 2 | 3 | 4 | 5 |
| 15. I believe that I was physically abused. | 1 | 2 | 3 | 4 | 5 |
| 16. I had the perfect childhood. | 1 | 2 | 3 | 4 | 5 |
| 17. I got hit or beaten so badly that it was noticed by someone like a teacher, neighbour, or doctor. | 1 | 2 | 3 | 4 | 5 |
| 18. I felt that someone in my family hated me. | 1 | 2 | 3 | 4 | 5 |
| 19. People in my family felt close to each other. | 1 | 2 | 3 | 4 | 5 |
| 20. Someone tried to touch me in a sexual way, or tried to make me touch them. | 1 | 2 | 3 | 4 | 5 |
| 21. Someone threatened to hurt me or tell lies about me unless I did something sexual with them. | 1 | 2 | 3 | 4 | 5 |
| 22. I had the best family in the world. | 1 | 2 | 3 | 4 | 5 |
| 23. Someone tried to make me do sexual things or make me watch sexual things. | 1 | 2 | 3 | 4 | 5 |
| 24. Someone molested me. | 1 | 2 | 3 | 4 | 5 |
| 25. I believe that I was emotionally abused. | 1 | 2 | 3 | 4 | 5 |
| 26. There was someone to take me to the doctor if I needed it. | 1 | 2 | 3 | 4 | 5 |
| 27. I believe that I was sexually abused. | 1 | 2 | 3 | 4 | 5 |
| 28. My family was a source of strength and support. | 1 | 2 | 3 | 4 | 5 |

**APPENDIX C**

Outcome modulating factor (OMF) questionnaires: the Connor-Davidson Resilience Scale (CD-RISC), the

Rosenberg Self-esteem Scale (R-SES), and the Edinburgh Handedness Inventory (EHI)

**CD-RISC**

Participant initials: _____     Participant number: _____     Date: _____

| Please indicate how much you agree with the following statements as they apply to you over the last **month**. If a particular situation has not occurred recently, answer according to how you think you would have felt. | not true at all | rarely true | sometimes true | often true | true nearly all the time |
|---|---|---|---|---|---|
| 1. I am able to adapt when changes occur. | | | | | |
| 2. I have at least one close and secure relationship which helps me when I am stressed. | | | | | |
| 3. When there are no clear solutions to my problems, sometimes fate or God can help. | | | | | |
| 4. I can deal with whatever comes my way. | | | | | |
| 5. Past successes give me confidence in dealing with new challenges and difficulties. | | | | | |
| 6. I try to see the humorous side of things when I am faced with problems. | | | | | |
| 7. Having to cope with stress can make me stronger. | | | | | |
| 8. I tend to bounce back after illness, injury, or other hardships. | | | | | |
| 9. Good or bad, I believe that most things happen for a reason. | | | | | |
| 10. I give my best effort, no matter what the outcome may be. | | | | | |
| 11. I believe I can achieve my goals, even if there are obstacles. | | | | | |
| 12. Even when things look hopeless, I don't give up. | | | | | |
| 13. During times of stress/crisis, I know where to turn for help. | | | | | |
| 14. Under pressure, I stay focused and think clearly. | | | | | |
| 15. I prefer to take the lead in solving problems, rather than letting others make all the decisions. | | | | | |
| 16. I am not easily discouraged by failure. | | | | | |
| 17. I think of myself as a strong person when dealing with life's challenges and difficulties. | | | | | |
| 18. I can make unpopular or difficult decisons that affect other people, if it is necessary. | | | | | |
| 19. I am able to handle unpleasant or painful feelings like sadness, fear and anger. | | | | | |
| 20. In dealing with life's problems, sometimes you have to act on a hunch, without knowing why. | | | | | |
| 21. I have a strong sense of purpose in life. | | | | | |
| 22. I feel in control of my life. | | | | | |
| 23. I like challenges. | | | | | |
| 24. I work to attain my goals, no matter what roadblocks I encounter along the way. | | | | | |
| 25. I take pride in my achievements. | | | | | |

**R-SES**

Participant initials: _____     Participant number: _____          Date: _____

**Instructions:**

Circle the appropriate number for each statement depending on whether you strongly agree, agree, disagree, or strongly disagree with it.

|  | 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 other 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'm a person of worth, at least on an equal plane 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 |

**EHI**

Participant initials: _____     Participant number: _____          Date: _____

| | Left | Right |
|---|---|---|
| 1. Writing | ☐ ☐ | ☐ ☐ |
| 2. Drawing | ☐ ☐ | ☐ ☐ |
| 3. Throwing | ☐ ☐ | ☐ ☐ |
| 4. Scissors | ☐ ☐ | ☐ ☐ |
| 5. Toothbrush | ☐ ☐ | ☐ ☐ |
| 6. Knife (without fork) | ☐ ☐ | ☐ ☐ |
| 7. Spoon | ☐ ☐ | ☐ ☐ |
| 8. Broom (upper hand) | ☐ ☐ | ☐ ☐ |
| 9. Striking Match (match) | ☐ ☐ | ☐ ☐ |
| 10. Opening box (lid) | ☐ ☐ | ☐ ☐ |
| **TOTAL(count checks in both columns)** | ☐ ☐ | ☐ ☐ |
| **Difference** | **Cumulative TOTAL** | **Result** |

**APPENDIX D**

Participant assessment sheet

**Participant assessment sheet**

Participant number: …………………………………………………

Name and Surname: …………......................................................

Cell phone number: …………………………………………………

Date: ……………………………………………………………………

**Girls**

1. Are you currently on hormonal contraceptives (circle yes or no)?................................**Yes/No**

2. Which day in you menstrual cycle is it today? Or, how many days ago was the start of your period?
……………………………………………………………………………………………………………………
…………………….

**Boys and girls**

1. In what type of exercise do you participate more than twice per week? (e.g., running, swimming, lifting weights, playing rugby/soccer, dancing, ect.
……………………………………………………………………………………………………………………
………………………………….

2. Do you participate in competitive sport (i.e. do you compete in matches/competitions)? If yes, provide detail please.
……………………………………………………………………………………………………………………
………………………………….

3. If you walk regularly, indicate in kilometres or time, how far you walk daily:
……………………………………………………………………………………………………………………
………………………………….

4. When last did you exercise? What exactly did you do?
……………………………………………………………………………………………………………………
……………………………………………………………………………………………………………………
……………………………………………………………………………….

5. Are you currently on any form of medication and if yes, for what condition are you taking medication?......................................................................................................................................

6. Are you currently suffering from any type of illness, for example, a common cold? If yes, please state the name of the disease or the symptoms you are experiencing:
……………………………………………………………………………………………………………………
……………………………………………………………………………………………………………………
……………………………………………………………………………………………………………………

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**APPENDIX E**

Determination of white blood cell specific glucocorticoid receptor (GR) expression

GR expression was assessed on the FASCAria flow cytometer. The GR quantification assay was optimized by means of piloting different permeabilization buffers as well as different concentrations of antibodies. In order to properly gate and identify cells, single stains, Fluorescence Minus One (FMO) controls and compensation - the process by which the fluorescence "spillover" originating from a fluorochrome other than the one specified for a particular Photomultiplier tube (PMT) detector is subtracted as a percentage of the signal from other PMT's – were also performed in advance of the blood draws, as well as after arrival of new antibodies. Trouble shooting to determine whether the prepared samples should be analyzed on the same day of preparation or could be analyzed the following day, was also performed. Cytometer setup and tracking (QC) beads were run each time samples were being analyzed and compensation was performed at least once a week. An unstained control for each participant was included.

The following protocol was followed:

**Materials:**

a) Permwash staining buffer

b) Fixation solution

c) FasLyse buffer

d) Distilled water

e) 4% Paraformaldehyde

**Preparations in advance:**

a) Dilute Staining buffer 1 in 10 (5ml SB in 50 ml distilled water)

b) Dilute Faslyse buffer 1 in 10

**Procedure:**

a) Label reaction vials for each participant, including an unstained control

b) Add 100 μl whole blood to every reaction vial as well as 1400 μl Faslyse – wait about 5 min

c) Make up a cocktail with panel of antibodies (n = number of participants):

*e.g. n = 5*

*CD3: (2 μl x n) = 10 + 1 = 11*

*CD19: (2 μl x n) = 11*

*CD45: (2 μl x n) = 11*

*CD14: (2 μl x n) = 11*

*CD56: (2 μl x n) = 11*

*= Total 1 (add to tube) = 55*

*Need 50 μl per person + 10 % pipetting error*

*N x 50 μl +10 % = Total 2*

*= 250 + 25 =275*

*Total 2 – Total 1 = μl Staining buffer (add to tube)*

*= 275 – 55 = 220*

d) Make up GR containing buffer (n = number of participants):

*e.g. n = 5*

*GR antibody: 2 μl x n = 11*

*= Total 1 (add to tube)*

*Need 50 μl per person + 10 % pipetting error*

*N x 50 µl +10 % = Total 2*

*= 250 + 25 = 275*

*Total 2 – Total 1 = µl Staining buffer (add to tube)*

*= 275 – 11 = 264*

e) Spin lysed samples at 600g for 5min

f) Throw off supernatant and add 50 µl Ab cocktail to each reaction vial except for unstained control – resuspend after each addition and vortex

g) Incubate for 30 min at 4°C

e) Wash with 500 µl Permwash buffer – centrifuge at 300g for 5 min

f) Throw off the supernatant and wash again with 500 µl Permwash buffer

g) Throw off the supernatant and add 250 µl fixation solution – resuspend

e) Incubate for 20 min at 4°C

f ) Wash twice with 500 µl Permwash buffer

g) Throw off the supernatant and add 50 µl GR-antibody containing buffer to all reaction vials accept for the unstained control

h) Incubate for 30 min at 4°C

i) Wash twice with 500 µl Permwash buffer

j) Throw off the supernatant and add 500 µl Paraformaldehyde

**APPENDIX F**

Trier Social Stress test (TSST) psychometric assessments: The State Anxiety version of the State-Trait Inventory (STAI-S) and the Visual Analogue Scale (VAS) for anxiety

**STAI – S / Part 1**

Participant initials: _____     Participant number: _____     Date: _____

Instructions:
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 feel <u>right now</u>, that is, <u>at this moment</u>. There are no right or wrong answers. Do not spend too much time on any statement, but give the answer which seems to describe your present feelings best.

| | **Not at all** | **Somewhat** | **Moderately so** | **Very much so** |
|---|---|---|---|---|
| 1.  I feel calm. | 1 | 2 | 3 | 4 |
| 2.  I feel secure. | 1 | 2 | 3 | 4 |
| 3.  I am tense. | 1 | 2 | 3 | 4 |
| 4.  I feel strained. | 1 | 2 | 3 | 4 |
| 5.  I feel at ease. | 1 | 2 | 3 | 4 |
| 6.  I feel upset. | 1 | 2 | 3 | 4 |
| 7.  I am presently worrying over possible misfortune. | 1 | 2 | 3 | 4 |
| 8.  I feel satisfied. | 1 | 2 | 3 | 4 |
| 9.  I feel frightened. | 1 | 2 | 3 | 4 |
| 10. I feel comfortable. | 1 | 2 | 3 | 4 |
| 11. I feel self-confident. | 1 | 2 | 3 | 4 |
| 12. I feel nervous. | 1 | 2 | 3 | 4 |
| 13. I am jittery. | 1 | 2 | 3 | 4 |
| 14. I feel indecisive. | 1 | 2 | 3 | 4 |
| 15. I am relaxed. | 1 | 2 | 3 | 4 |
| 16. I feel content. | 1 | 2 | 3 | 4 |
| 17. I am worried. | 1 | 2 | 3 | 4 |
| 18. I feel confused. | 1 | 2 | 3 | 4 |
| 19. I feel steady. | 1 | 2 | 3 | 4 |
| 20. I feel pleasant. | 1 | 2 | 3 | 4 |

**VAS for anxiety**

Participant initials: _____          Participant number: _____          Date: _____

**Instructions:**

On a scale of 0 to 10, please tell me your current level of anxiety. A mark at 0 would be no anxiety, 5 would be moderate anxiety, and 10 would be severe anxiety.
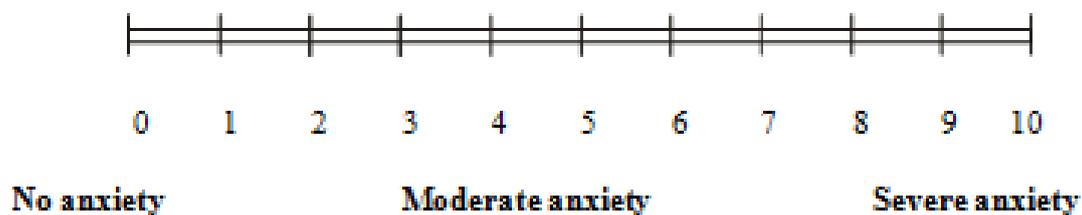
**APPENDIX G**

Trier Social Stress test (TSST) for groups (n = 3 - 8): procedure and scripts

**TSST procedure**

**Set-up**

a. Committee

• Experimenter 1 (E1): E1 is responsible for guiding the subject from one room to another and debriefing the subject.

• Confederates (Cs): Two confederates are required. Neither should have had contact with the participants prior to the TSST.

> ➢ Confederate 1 (C1): C1 will be the only person to speak to the participants during the TSST.
> ➢ Confederate 2 (C2): C2 is the only person to take notes during the procedure.

**b. Environment**

• Consultation room: This room should have comfortable chairs and bland reading material for the participants. The participants are placed in this room before and after the TSST. Paper and pencil/pen with a writing area (clipboard or small table) are available for writing.

• Testing room: Screens serving as mobile dividing walls between the participants are set up a day in advance. The testing Room should be a plain room containing a desk with two chairs behind it. This is the room in which the speech and math tasks are conducted. The video camera is set on a tripod behind the Cs.

**c. Materials**

• Paper, pencil and clipboard for each participant

• Lab coats for each confederate

• Questionnaires (VAS and STAI-S)

• Scripted material: Script with instructions for the job interview and mathematics task, and debriefing script

• Two timers with audible ticking and alarm

• Video camera

• Vacutainer tubes, absentee letters to the schools involved, and reimbursement money

**d. Procedure**

• Preparation period (30 min)

  ➢ Participants are to arrive between 08:00 and 08:30.

  ➢ The VAS and STAI questionnaires are administered.

  ➢ Blood is drawn from each of the participants by a research nurse.

• TSST (30min)

Phase I (10min): Preparation of job interview

  ➢ E1 provides the participants with written instructions regarding the preparation of an application for a job of his/her choice, and reads the instructions out loud with them. They are told that a video recording of their 2 min speech will be conducted and that the panel could come back to them at any time throughout the procedure to ask further questions. The written instructions end with the mathematics task that will follow the speech. If the participants ask E1any questions regarding the task, the response is: "Do whatever you think is best" or "I do not know any other details."

  ➢ After reading the instructions, the participants are given 10min to prepare, and are provided with a pencil and paper to outline their speech (although they may not take it into the testing room).

  ➢ E1 leaves the room for 10 minutes.

Phase II (12min): Public speaking task

  ➢ Participants are guided to the testing room by E1 and asked to stand in a row in front of the committee, who are sitting behind a table with a video camera and stop watch.

  ➢ E1 gives a short verbal summary of the forthcoming task and leaves the room. Participants are separated by mobile dividing walls to prevent any eye and social contact with each other.

➢ C1 does all the talking and calls on each of the participants in a random fashion to start their speech. The two Cs should withhold verbal and non-verbal feedback during the speech and maintain eye contact with the participant delivering a task.

➢ The timer is set to 2min and C1 tells the selected participant to begin. Whenever a participant finishes in less than two minutes or pauses, Gareth responds with "You still have some time left. Please continue!". If the participant asks the Cs a question, C1 makes neutral comments, such as "Do whatever you think is best," "Say whatever comes to your mind," or "Be as creative as you like."

➢ C2 takes notes approximately every one minute, as if noting the subject's performance. The comments should be brief so that Melanie's eyes are not taken off the participant for more than a glance.

➢ When the alarm sounds, C1 says "Please stop, your time is up."

Phase III (8min): Mental arithmetic task

➢ After all participants have given their speeches, C1 asks the participants individually to serially subtract the number 16 from a given number as quickly and accurately as possible. Each participant has his/her own individual starting number. For example, C1 tells the selected participant: "Now we would like you to subtract number 16 from 6233, and keep subtracting 16 from the remainder until we tell you to stop. You should do the subtraction as fast and as accurately as possible."

➢ Each participant is allowed 80s of calculating.

➢ If they make a mistake, they have to restart at their personal number when Gareth interrupts with: "Stop. Please start again". If the participant has forgotten the starting number, Gareth provides the number again.

➢ At the end of the 80s, C1 instructs the subject "Please stop, your time is up. You can go back to the consultation room now." If the participant asks questions as to how he/she did, C1 responds "I am not allowed to tell you that. Someone will give you that information later."

Adverse Response: If at any time the participant appears to be having an adverse reaction, i.e. begins to cry or seems overly agitated, C1 would ask: "Are you okay?" "Do you want to stop?" or "Are you okay to continue?" If the participant indicates that they wish to stop, C1 should stop the task at hand immediately and notify E1 so that the participant can be debriefed.

• Resting and debriefing period (60min)

> ➤ After the participants have gone back to the consultation room, the VAS is re-administered.

> ➤ A final blood draw is obtained and the participants are then debriefed by E1 (by means of reading a script).

**TSST scripts**

**a. Instructions**

You will do two tasks today: firstly, there will be two trained interviewers in another room who will assess how outgoing, gregarious, and comfortable you are in situations in which you must project yourself as an expert. This is a type of personality test for a trait called extraversion. You will be given a hypothetical situation in which you will be applying for your ideal job. You have dreamed about working in this job for as many years as you can remember. You have just seen an advertisement for this perfect job and decided to apply. After submitting your application, you have been invited for an interview. The job pays a very large salary. You are competing against a lot of other candidates, and the final selection will be made based on your ability to convince the interviewers of how your experiences, abilities, and education make you a better candidate that the others. You will try to convince this panel of interviewers that you are the best candidate for the position. The panel could come back to you at any time throughout the procedure to ask further questions. You have 2 minutes to do this.

You will have 10 minutes to prepare this detailed speech. After the preparation time has elapsed, you will deliver your speech to the interviewers. Your speech should explain why you should get the job. Remember, you should try to perform better that all of the other participants. These examiners are specially trained to monitor and rate your speech for its believability and convincingness, and they will compare your performance to that of the others who perform this task. Also, you will be videotaped during the task so that the examiners can go over the videotape carefully and rate the contents of you speech as well as your nonverbal behaviour. In addition, you will be asked to perform a mental math test, which will give us additional information about your working memory capacity.

Now you have 10 minutes to prepare for your 2 minute job interview. You will be provided with a pencil and paper to outline your speech, although you may not take it into the test room.

**b. Debriefing**

You were not actually being evaluated or scored. You were not actually being recorded. Your performance is not compared to other participants. We are measuring a naturally occurring stress hormone in the body called cortisol. We wanted to see what happens to this hormone in your body under stress, that's why we have been collecting blood samples from you. We are sorry that we didn't tell you the truth about everything, but if we had, the situation wouldn't have been stressful. You did a good job. Thank you for participating. Do you feel okay to go home or leave?