Title

Early-life adversity and adulthood psychological distress: The role of biological stress reactivity

Permalink

https://escholarship.org/uc/item/3vk4j97r

Author

Goldman-Mellor, Sidra Jessie

Publication Date

2012

Peer reviewed|Thesis/dissertation

Early-life adversity and adulthood psychological distress:
The role of biological stress reactivity

By

Sidra Jessie Goldman-Mellor

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Epidemiology

in the

Graduate Division

of the

University of California, Berkeley

Committee in Charge:

Professor Ralph Catalano, Chair
Professor Darlene Francis
Professor Alan Hubbard

Spring 2012

Early-life adversity and adulthood psychological distress: The role of biological stress reactivity

by Sidra Jessie Goldman-Mellor

ABSTRACT

Early-life adversity and adulthood psychological distress:
The role of biological stress reactivity

by

Sidra Jessie Goldman-Mellor

Doctor of Philosophy in Epidemiology

University of California, Berkeley

Professor Ralph Catalano, Chair

Background. Social epidemiology has become increasingly interested in the effect of early-life experiences on health outcomes in adulthood. It is now widely recognized that early-life adversity (such as being abused or neglected) is an important risk factor for adulthood depression and other psychological disorders. Research emerging from the developmental programming paradigm posits that suboptimal early-life environments can encode vulnerability to later-life psychological impairment through permanent alterations in biological systems, particularly the neuroendocrine and immune systems. However, a subset of persons exposed to early-life adversity appear to be psychologically resilient, experiencing no or very minimal mental health problems later in life. It is a critical goal in epidemiology to ascertain whether these distinct life-course psychological trajectories are mediated by distinct biological characteristics. In this dissertation, I used data from a unique cohort study to explore the relationship between participants' early adversity exposure, their longitudinal history of psychological distress, and their neuroendocrine and immunological stress reactivity systems.

Methods. The dataset comprised a subsample of the ongoing Whitehall II occupational epidemiologic cohort study. The subsample was a group of healthy older male and female Whitehall II members (n = 543) who participated in the 2008 Heart Scan Study (HSS), which was primarily designed to investigate the association between physiological reactivity to an experimental laboratory stressor and sub-clinical coronary artery calcification. The Heart Scan Study data were subsequently linked to six waves of the participants' previously collected Whitehall II study data (spanning years 1985-2004).

I defined exposure to early-life adversity using a composite variable derived from questions collected during both the Heart Scan Study and in one Whitehall II wave. Participants were categorized as exposed if they reported experiencing physical abuse, parental death, separation from their mother or time spent in an orphanage for 1+ years, serious familial mental illness or substance abuse, parental divorce, frequent parental conflict, and/or a harsh parenting style before the age of 16. Participants' history of psychological distress during adulthood was derived from their Whitehall II data, which included General Health Questionnaire-28 (a measure of psychiatric symptoms) scores at each wave. I used two of the physiological reactivity parameters measured in the HSS – cortisol (a key stress hormone) and interleukin-6 (an important inflammatory protein marker) – as my outcome variables.

First, I used piecewise multilevel growth curve modeling to examine whether study participants' joint early-life adversity and psychological history status – i.e., early-life adversity exposure (yes/no) and recurrent psychological symptoms (yes/no) – predicted mean

differences in their cortisol response trajectories. Second, I used the mixture model technique of group-based trajectory modeling to identify whether distinct clusters of stress-reactivity patterning existed within cortisol and interleukin-6, and if so, whether the clustering pattern within one biomarker was associated with clustering in the other. Lastly, I used an extension of group-based trajectory modeling to determine whether early-life adversity and longitudinal history of psychological distress predicted study members' membership in those clusters.

Results. Using the piecewise multilevel growth curve analysis, I found that participants exposed to early-life adversity had dysregulated cortisol reactivity trajectories, but that the pattern of those trajectories differs based on their history of psychological distress during adulthood. Adversity-exposed adults with a history of <u>recurrent</u> psychological distress had blunted cortisol responses compared to non-adversity-exposed adults, as well as decreased overall cortisol output. In contrast, adversity-exposed adults with <u>minimal</u> psychological distress had elevated levels of cortisol at baseline and prolonged responses to stress. There was also some evidence that this association was modified by gender.

Using group-based trajectory modeling, I found that distinct clusters did appear to exist within both the cortisol and IL-6 data, although these results need to be interpreted with extreme caution due to statistical flaws in the modeling approach. The four cortisol clusters were distinguished from each other by both mean baseline cortisol levels as well as slightly different reactivity patterns during the experimental laboratory session; the clusters corresponded to a "low-declining" group, a "middle-flat" group, a "higher-flat" group, and finally a "high-declining" group. Within the IL-6 data, the fitted solution comprised five trajectory groups, all of which shared a similar growth pattern (steadily increasing) but had slightly different mean baseline values. The cortisol and IL-6 trajectory groups appeared to be moderately correlated with each other, such that "opposite" biomarker groups had a slight tendency to associate together; however, the principal finding from this dual trajectory analysis was that membership in a given cortisol or IL-6 group is not a very sensitive predictor of group membership in the other biomarker.

In the last piece of analysis, I found that adversity-exposed individuals who subsequently had <u>recurring</u> psychological distress symptoms were marginally more likely to fall into the lowest cortisol trajectory group. Otherwise, cortisol and IL-6 trajectory group membership was not significantly predicted by any combination of early adversity exposure and psychological distress history. Participants' age, body mass index, and socioeconomic status did predict both cortisol and IL-6 group memberships.

Conclusions. My findings partially support the hypothesis that different patterns of atypical stress reactivity – in this case, blunted vs. heightened cortisol responses – may be encoded in the aftermath of early-life adversity, and that these patterns in turn may promote divergent susceptibilities to lifetime psychological disorder. Inflammatory stress reactivity, as measured by interleukin-6, does not appear to play a role in this association. However, distinct clusters of stress reactivity patterning do appear to exist within cortisol and interleukin-6, and may be predicted by theoretically important sociodemographic variables, an observation that should be explored in future research. Although is unclear how much of the burden of depression/anxiety symptoms the observed cortisol patterns might explain among adversity-exposed populations, my findings do provide support for the notion that social experiences "get under the skin" and contribute to population ill-health. This work may lead to a better understanding of the biological sequelae of early-life adversity, and the effects those sequelae can have on psychological risk and resilience throughout the lifecourse.

This dissertation is dedicated to my beloved husband, Alex Mellor, who gave me the best possible reason to journey to England and pursue this research in the first place. Throughout the past five years – even the two when we were more than five thousand miles apart – Alex provided the constant encouragement, support, laughter, and sense of perspective I needed to survive the often difficult process of pursuing a PhD. I am eternally grateful for his willingness to embrace an entirely new life with me in a new country, and his abiding love throughout our adventures together have made these years my happiest yet.

I also dedicate this dissertation to my parents, Alvin Goldman and Holly Smith, who set an unparalleled example throughout my life for intellectual rigor, academic service, and the rare privilege of getting to do a job you love. I am deeply grateful for their commitment to my education, for their tough questions, and for their everlasting support and love.

Table of Contents

Acknowledgments

I would first and foremost like to thank Dr. Andrew Steptoe for affording me the extraordinary opportunity to work with him and his research group at University College London. Dr. Steptoe graciously hosted me at UCL for two summers, allowed me to help with the Heart Scan Study data collection, and subsequently encouraged me to use the data for my dissertation research. Throughout that process he has served as a invaluable mentor, and I am tremendously grateful for his generosity, his guidance, and his time.

I also thank my committee chair and academic advisor at Berkeley, Dr. Ray Catalano, who provided the best possible example of what it is to be a scientist and a mentor, and inspired me to "push back the frontiers of ignorance" a little farther. Dr. Catalano's dedication to his students is truly remarkable. Throughout my years of research with him, he has constantly challenged my assumptions, provoked new questions, and inspired me with his deep integrity and broad sense of perspective about the scientific endeavor. I am so thankful for the countless hours he spent improving my skills as a researcher and a writer, the opportunities he gave me to work on fascinating questions with a brilliant team of scientists, and last (but certainly not least) his generous funding and logistical support. Every PhD student should be lucky enough to have such an advisor.

Dr. Alan Hubbard and his biostatistics PhD student, Luca Pozzi, provided invaluable statistical advice and assistance, and pushed me to go beyond the status quo in my dissertation methodology. I am particularly grateful to Luca, who spent many hours perfecting my code, talking through my analytic strategy, running crucial analyses, and generally supplying advice and insight. I could not have done this without him.

Dr. Darlene Francis was the first person to kindle my interest in how social experiences "get under the skin" to cause health problems, and she has provided brilliant inspiration throughout this process with her probing questions and unique perspective.

I would also like to thank Dr. Len Syme, who originally recruited me to Berkeley, facilitated my work with Dr. Steptoe, and has served as a steadfast mentor and guiding light in his dedication to alternately challenging me and encouraging me when I most needed it; Dr. Jennifer Ahern, who has generously provided invaluable feedback and mentorship at crucial stages; and Dr. Ira Tager, whose rigorous and thoughtful methodological training provided the foundation for all my epidemiologic skills and interests.

I thank all my friends and fellow students – especially Claire Margerison-Zilko, Jennifer LaChance, Elizabeth Carlton, Mina Subbaraman, Caitlin Gerdts, and Katherine Saxton – whose support and epidemiologic brilliance was essential to making my dissertation work and life as a PhD student the rich and rewarding experience it has been. I hope I have brought them even a tiny fraction of the joy, laughter and comradeship that they have brought me.

Lastly, thank you to Raphe Goldman, Sarah Crow, and Fiona and Jacob. I am eternally grateful for these past seven years of delicious dinners, playtime, help and support, an occasional shoulder to cry on, and the countless, unending joys of family. You are a constant inspiration.

Chapter 1: Introduction and Background

1.0 Background to the Dissertation

Over the last twenty years, the field of social epidemiology has become increasingly interested in the effect of early-life experiences on health outcomes in adulthood. As part of the burgeoning discipline of "life course epidemiology," such research on the lasting sequelae of early-life experiences has particularly focused on chronic disease outcomes [1]. Much of the initial impetus for this body of research stemmed from a series of seminal studies in the 1990s by David Barker and colleagues, who used retrospective cohort data to report that individuals' risks for coronary artery disease, diabetes, respiratory disease, and other poor outcomes in adulthood were predicted by markers of their prenatal and infant growth – signals of early undernutrition [2, 3].

Around the same time, psychiatric epidemiologists were beginning to more systematically examine the effect of early-life experiences on later psychological illnesses. Although it had long been recognized that exposure to early adversity placed individuals at greater risk of depression and other disorders, methodological limitations of early studies prevented clear inference about the nature of this association [4]. This led to the implementation of several large-scale, population-representative, cross-sectional epidemiological studies investigating the relationship between self-reported childhood adversity and psychological disorder in adulthood [4-6].

These studies firmly established that exposure to childhood adversities – such as sexual abuse, physical abuse, neglect, parental psychopathology, or parental death – greatly increased risk for psychological problems, particularly mood disorders such as clinical depression. Odds ratios for this relationship typically range between 2.0 and 10.0, depending on the specific exposure and outcome. More recent studies, some of them longitudinal and/or from international settings, have confirmed and extended these findings. It is now widely recognized that early-life adversity significantly predicts depression and other psychological disorders. Importantly, mood and anxiety disorders among early adversity-exposed individuals very often manifest in early-onset, severe, and highly recurrent forms compared to such disorders in non-adversity-exposed persons [7-12]. Childhood adversities also significantly predict various physical health outcomes, although the magnitude of these associations are generally smaller, with odds ratios ranging from 1.2 to 3.9 (e.g., [6, 13, 14]).

The consistency and strength of the association between experiencing adversity in early life (also termed "early-life stress," or ELS) and mood disorders, as well as growing awareness about the massive public health burden stemming from such disorders, has resulted in an enormous proliferation of multi-disciplinary research investigating the mechanisms that underlie this relationship. A large proportion of this research has focused on identifying the *biological* mechanisms that may be operating, with one aim as someday being able to intervene upon the relevant biological systems and thereby prevent or treat adverse psychological sequelae of ELS [15-17].

At the same time, a separate field of research – the resilience literature – has established that as many as 60 percent of individuals exposed to ELS do *not* experience elevated risk for psychological disorder during their lifetimes ([18]; see below). However, most current research examining the biological mechanisms connecting ELS to psychological disorder ignores this population variability, and rarely attempts to distinguish biological effects

observed in ELS-exposed persons *with* psychological disorder from those observed in ELS-exposed persons *without* psychological disorder. In order to infer a causal relationship between exposure to ELS and the ensuing development of risk or resilience to psychological disorder, it will be critical to ascertain whether these distinct life-course psychological trajectories are associated with distinct biological pathways. This goal provides the motivation for my doctoral dissertation research.

1.2 Key Definitions

Prior to providing a detailed overview of the literature investigating the biological pathways underlying the association between early-life stress and psychological disorder, it will be helpful to supply working definitions of key terms that will be used throughout this dissertation.

Stress. "Stress" is an exceedingly broad term that has long been criticized for its vagueness and incorporation of multiple concepts from diverse fields. In the context of this dissertation, I will use the conceptual definition proposed by Cohen, Kessler and Underwood [19] and widely used, which defines stress as a process that encompasses an environmental stimulus/demand, an individual's appraisal of that stimulus as demanding or threatening, and an ensuing response (physiological or behavioral). An overview of the physiological responses provoked by stress are described in more detail below.

Early-Life Stress. Likewise, "early-life stress" can be defined in multiple ways, and the literature includes a variety of interpretations, some more expansive than others. In the context of this dissertation, early-life stress refers to exposure to *one or more categories* of significant adversity during childhood or adolescence (ages 0-15 years, approximately). These categories include outright maltreatment, such as physical, sexual, and emotional abuse or physical neglect, as well as less easily classified adversities such as experiencing the death of a parent, parental divorce, domestic violence, parental mental illness or substance abuse, or family environments generally characterized by aggression, conflict, and cold or unaffectionate interaction styles [7, 20-23]. The severity and duration of such stressors varies. However, evidence suggests that even exposure to relatively mild forms of ELS can increase risk for psychological problems [24]. There is also support for the concept of "equifinality" [25], in that different forms of ELS lead to similar outcomes – in other words, specific categories of ELS do *not* appear to be associated with specific psychological disorders.

Mood and anxiety disorders. Using the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) classification, "mood disorders" refers to a group of diagnoses encompassing major depressive disorder (MDD), dysthymia, and depressive disorder not otherwise specified (DD-NOS). Anxiety disorders include generalized anxiety disorder (GAD), social anxiety disorder, specific phobia, panic disorder with and without agoraphobia, obsessive-compulsive disorder (OCD), posttraumatic stress disorder (PTSD), anxiety secondary to medical condition, acute stress disorder (ASD), and substance-induced anxiety disorder. It should be noted that while much of the research on ELS and psychological disorders uses *clinical diagnoses* of these disorders, other studies – including the work presented in this dissertation – instead use measures of sub-clinical disorder or non-specific psychological distress symptoms. The history of psychology and psychiatric epidemiology includes many debates about the relevance, clinical or otherwise, of such sub-clinical symptoms. A full discussion of this topic is beyond the scope of this dissertation. As sub-clinical psychological distress is a legitimate concern in its own right, both as an important

source of population morbidity [26, 27] and as a risk factor for various health outcomes including coronary heart disease [28], my working definition of mood and anxiety disorders includes cases of such sub-clinical symptoms.

  <u>Resilience.</u> The study of resilience, like the study of stress, has a long and complex history; the term's definition has evolved over time and is still debated. A fairly simple and much-cited recent definition, from Luthar, Cicchetti et al. [29], describes resilience as a "process through which positive adaptation is achieved in the context of adversity." As with early-life stress, the construct of resilience can include multiple components, including those of time, level of risk or adversity experienced, and domains of competence (e.g., psychological, social, academic, etc.) [30]. Although early definitions of resilience viewed it as a stable personal characteristic, resilience is now recognized to be a dynamic process that can change over time and reflects "ongoing transactions between a child and the environment" [31]. In my dissertation, "resilience" will primarily refer to psychological resilience, i.e., positive psychological adjustment (specifically, low or nonexistent symptoms of mood/anxiety disorder, generally measured over multiple time points) among persons exposed to ELS.

1.3 Early-Life Stress and Mood/Anxiety Disorder: Magnitude of the Problem

  Although early-life adversity has only recently been identified as a risk factor for poor health outcomes, and awareness of its effects is fairly limited outside academic circles [32], evidence suggests that it is a significant public health problem. The same can also be said for mood and anxiety disorders, which along with other mental illnesses have historically been given less attention and funding than somatic illnesses. An overview of the epidemiology of ELS and mood/anxiety disorders, as well as the association between them, is provided in the paragraphs below.

1.3.1 Early-life stress is highly prevalent

  There is a limited number of estimates of the population incidence of early-life stress, and most of those available rely entirely on self-reported information collected during adulthood. However, several of these estimates are derived from methodologically rigorous epidemiologic studies, increasing their reliability and validity. All estimates consistently find that exposure to ELS is extremely common.

  In the U.S., researchers using the 1990-1992 National Comorbidity Survey (NCS) and, later, the 2001-2003 NCS Replication Sample (population-representative surveys of the entire country), found that between 53.4% and 74.4% of respondents reported experiencing at least one of a range of adversities between the ages of 0 and 18 years [4, 33]. In the original NCS, 23.2% of respondents reported experiencing one adversity, 16.1% experienced two, and 35.0% experienced three or more adversities [4]. In that sample, the most commonly experienced adversity was maternal depression, at 22.7%; parental verbal aggression, paternal problems with alcohol or drugs, and parental separation or divorce were also very common (16.4-20.0%). In the NCS Replication Sample, the most common adversities had shifted somewhat: parental divorce (17.5%), family violence (14.0%), severe family economic adversity (10.6%) and parental mental illness (10.3%) were the most common [33].

  In a large combined retrospective and prospective study of >17,000 Kaiser Permanente plan members in San Diego, called the ACE Study, Felitti et al. [6] found that 52.1% reported one or more adverse childhood experiences. The most common experience was living with

someone who was a substance abuser (25.6%), but sexual abuse (22.0%) and household member mental illness (18.8%) were also very common. Even though this sample is largely middle-class, more than 13% of the sample reported three or more adversities [6].

Using a Baltimore-area representative sample, Menard et al. [34] found similarly striking results, with 43.4%-58.7% of the sample reporting exposure to one, two, or three adversities, 23.4%-41.2% reporting four to six adversities, and 0.7%-13.2% reporting seven or more adversities (the exact percentages varied according to respondents' race and welfare status). In this sample, emotional abuse, parental mental illness, and parental substance abuse were the most common adversities. In a sample of Memphis, Tennessee adults, the numbers were slightly lower: 35.1% of the sample had experienced at least one form of childhood maltreatment, and 13.5% experienced two or more [35]. In this sample, physical abuse, physical neglect, and emotional abuse were the most commonly experienced adversities [35]. (In both of these studies, as in Kessler et al. [4] and Felitti et al. [6], the construct of emotional abuse generally reflects verbal aggression, such as insults or threats of violence.)

Internationally, there are fewer studies, although the World Health Organization's (WHO) World Mental Health Survey provided a comprehensive and population-representative assessment using data from 21 countries. In that study, approximately 40% of respondents in all countries (across average income levels) reported *any* childhood adversity; approximately 15.5% reported three or more [36]. Once again, parental death, physical abuse, family violence, and parental mental illness were the most common categories. Approximately 8.6% of the participants in a longitudinal population-representative cohort in New Zealand were exposed to severe childhood maltreatment (measured both by external observers in childhood and by self-report in adulthood); a similar figure (7.6%) was reported for another longitudinal cohort of children in the U.K. [37]. While these numbers are considerably lower than those reported in U.S. samples, these studies did not include measures of several ELS categories used in other studies. In an international sample of adults from the United States, Australia, England, and the Netherlands, 39.5% of participants reported exposure to one or two adversities and another 32.9% reported experiencing three or more – and these participants had no current or prior diagnosis of major depression, anxiety, substance abuse, or neurological brain disorder [8], meaning that the true prevalence was likely underestimated.

Data on the demographic correlates of ELS are mixed, and require further study. Women appear to be more likely to experience any category of adversity, with the occasional exception of physical abuse and physical neglect [4, 6, 35, 38]. Older individuals, white and Asian individuals, and college graduates tend to be less likely to have experienced any ELS, and are exposed to fewer categories of adversity [6, 12, 35].

As is clear from the data described above, categories of ELS exposure are known to often cluster together. Although this relationship is probabilistic rather than deterministic – exposure to any given ELS category does not perfectly predict exposure to any other category – the majority of people who report exposure to one kind of adversity also report exposure to at least one other kind [4, 39-41]. In the NCS-Replication Sample, among respondents with more than one adversity, the mean number reported was 3.2 [33]. In the Adverse Childhood Experiences (ACE) study in San Diego, if one adversity was experienced, there was an 87% probability that at least one other adversity was also experienced [38]. In particular, people exposed to sexual abuse have been shown to experience, on average, four other types of adversity [4]; likewise, among those exposed to emotional or physical neglect, the probability of also reporting parental divorce, substance abuse, mental illness, and/or domestic violence is

between 30-50% [41]. Which adversities are most highly correlated varies with the sample. In the NCS survey, the most highly correlated adversities were parental psychopathologies [4]; in Baltimore, physical and emotional abuse as well as familial incarceration and substance abuse were the most frequent combinations [34]; in a representative Memphis sample, the most commonly co-occurring categories were emotional abuse and physical abuse, and physical abuse and physical neglect [35].

1.3.2 Mood and anxiety disorder are highly prevalent

As with early-life stress, mood and anxiety disorders – particularly major depression – are extremely prevalent, and often some of the most devastating of all health conditions. In the World Health Organization's World Mental Health Survey, mood and anxiety disorders were the most common mental disorders, with population lifetime prevalences ranging from 3.3% to 31.0%, depending on the country [42]. Within the U.S., using data from the NCS Replication Sample, researchers reported that the twelve-month prevalence for anxiety disorders was 18.1%; for mood disorders, it was 9.5% [43]. The disorders are very often comorbid: correlations between specific mood and anxiety disorders typically exceed .5, and approximately 58-70% of people with generalized anxiety disorder also meet criteria for major depression [43]. Forty-five percent of the mood disorder cases in the NCS-R were classified as serious, indicating that the individual either reported a serious suicide attempt in the last 12 months, work disability or substantial limitations due to their disorder, any bipolar disorder, or 30 or more days "out of role" in the year [43]. Married females, and those with a high education level, were more likely to have a mood or anxiety disorder. *Lifetime* prevalence estimates in the U.S. are considerably higher – nearly 30% of the sample was reported as ever having had an anxiety disorder, and 20.8% had ever had a mood disorder [44]. These disorders typically have an age of onset between 20 and 30 years and are often highly recurrent, presenting a significant lifetime burden to affected individuals [44, 45]. I note, however, that the NCS, NCS-R, and World Mental Health Surveys' findings are based on their use of the Composite International Diagnostic Interview (CIDI) rules, which index *inferred* rather than *diagnosed* or *treated* disorder. Controversy about the validity of CIDI-indexed disorder vs. clinician-diagnosed disorder remains (e.g., [46]).

Mood and anxiety disorders are also associated with significant health, economic and social burdens. The World Health Organization estimated in 1999 that major depression was the fifth leading cause of years lost due to disability (DALYs) worldwide; it projected that depression would become second only to cardiovascular disease in this regard by 2020 [47]. Mood and anxiety disorders are associated with increased functional impairment, lost work productivity, lower educational attainment, increased risk of teen parenthood, and higher medical costs [48, 49]. A recent review estimated that mood and anxiety disorders combined cost European countries an annual total of €187.8 billion, in direct and indirect costs [50]. Because these disorders are under-diagnosed, expensive to treat, and often resistant to available treatments, public health officials have emphasized that prevention of disorder is a critical goal [51, 52].

1.3.3 Early-life stress predicts mood/anxiety disorder

As cited above, exposure to ELS is a potent predictor of increased risk for mood and anxiety disorders. Current findings suggest that between 25% and 49% of people exposed to ELS will develop symptoms of depression and/or anxiety, depending on the sample [9, 53, 54].

In the National Comorbidity Sample and WHO World Mental Health Survey, researchers found that various early adversities were associated with 1.2- to 10.4-fold higher odds of disorder, even adjusting for sociodemographic characteristics and psychological comorbidity [4, 36]. Parent marital break-up and repeated rape were especially strong predictors of disorder, and although adversities often clustered, even individual adversities were associated with significantly increased risk. Odds ratios for the association between ELS and depression/anxiety in other studies are of similar magnitude, although estimates vary as to which kinds of adversities are most strongly correlated with disorder [4, 9-12, 53-56]. Generally, an increasing number of adversities is associated with increased likelihood of disorder, in a consistent dose-response relationship [4, 9, 11, 12, 53]. The NCS-R results, however, indicated that although the odds of disorder increased with an increasing number of adversities, it was a non-additive effect – in other words, the odds increased at a decreasing rate [33]. There may be some sort of "threshold" effect past a certain number of adversities.

Importantly, many studies, including a recent meta-analysis, found that mood and anxiety disorders among ELS-exposed individuals tend to manifest in comparatively severe, early-onset, highly recurrent, and treatment-resistant forms [57]. Widom et al. [9] reported that documented exposure to childhood abuse and neglect predicted higher risk for current and lifetime major depression (ORs 1.59-1.75) in young adulthood; in addition, compared to controls, individuals who had been abused and neglected exhibited earlier age-of-onset for major depression (by an average of 2.6 years) and had higher rates of comorbidity. Similarly, Slopen et al. [12] found that in a representative South African national sample, experiencing childhood adversities was strongly associated with psychological disorder, with onsets more likely to be in childhood and adolescence or young adulthood. Using a large sample of depressed patients from the Netherlands, Wiersma et al. [11] reported that lifetime chronicity of depression was associated with a significantly higher prevalence of childhood trauma. In the San Diego ACE study, a higher ELS score predicted higher rates of chronic depression and suicide attempts [38].

Cohen et al. [8] found that increasing severity of current emotional distress (i.e., depression/anxiety symptoms) was related to higher total number of reported adverse childhood experiences. Interestingly, ELS was associated with symptom severity even though this study used a sample of psychologically healthy adults. Korkeila et al. [56] also found evidence that ELS was associated with worse disorder severity: in their Finnish sample of working-aged adults, experiencing childhood adversities was associated with a 1.28-2.70-fold increase in the odds of depression, a 1.29-1.94-fold increase in the rate of antidepressant prescriptions, and a 1.17-4.04-fold increase in the risk of hospitalization due to depression. Although many of the cited studies examined these effects in relatively young samples, the association appears to persist over the lifecourse. In a population-based sample of middle-aged and older adults, childhood adversity predicted current depression symptoms, with a similar effect size (OR=1.80) [55].

There are very few estimates of what percentage of the incidence of mood/anxiety disorder might be attributable to ELS. However, those that exist are fairly rigorous, and offer striking evidence that ELS is responsible for a large proportion of disorder. Data from the WHO World Mental Health Survey indicate that childhood adversities may be responsible for 23% of the incidence of mood disorders, 31% of the incidence of anxiety disorders, and nearly 30% of all measured psychological disorders (including mood, anxiety, substance use, and behavioral disorders) combined [36]. Similarly, estimates using the ACE sample found that

54% of current depression and 58% of suicide attempts in women could be attributed to childhood adversity [38, 58].

1.3.4 Psychological resilience to early-life stress

Although the main focus of this dissertation is not on psychological resilience to ELS, a brief overview of that literature's findings may help frame the discussion. In a recent comprehensive review, Vanderbilt-Adriance & Shaw [30] noted that rates of psychological resilience (low or nonexistent symptoms of mood/anxiety disorder) among abused, neglected, and otherwise at-risk children varied substantially by certain factors. In studies of predominantly white, middle-class children or children with only one category of risk, resilience rates tended to be higher – approximately 40-60% of the children did well with respect to psychological functioning. In studies of children from low socioeconomic status or multiple-risk contexts, resilience rates were lower. In those studies, only 1.5%-26% of children remained resilient. Notably, psychological resilience does not necessarily translate to resilience in other domains – children who are apparently psychopathology-free may still have significant problems with criminal behavior, social competence, educational attainment, or other functions [30]. Psychological resilience also fluctuates over time, with evidence that many individuals resilient at one point in time do not meet criteria for resilience at another time [30]. However, this literature is not large, and more longitudinal studies will be needed to provide rigorous rate estimates of persistent resilience.

1.4 Psychobiological Mechanisms Linking Early-Life Stress and Mood/Anxiety Disorder

It appears that different trajectories of psychological risk ensue in the population of children exposed to early-life stress: one trajectory leading to increased lifetime vulnerability to psychological disorders, another leading to resilience. The massive public health burden of psychological disorders and childhood adversities (independently and combined) indicates that there is a critical need to identify the mechanisms through which (a) ELS increases risk for psychological disorders, and (b) how these divergent outcomes emerge.

Researchers in epidemiology, psychology, and various biomedical science disciplines have responded to this need with an outpouring of research that attempts to identify the biological mechanisms through which ELS exposure increases risk for psychological disorders. Broadly speaking, much of this psychobiology research is based on the developmental programming paradigm first described by Barker and colleagues, which posits that suboptimal early-life environments can encode vulnerability to later-life psychological impairment through permanent alterations of organ systems and functioning (e.g., [20, 59]). Many organ systems and biological processes are implicated in this relationship, including brain structure changes, alterations in cognitive and emotional responding, behavioral functioning, and more (see [60-64] for more information). The conceptual and mechanistic models most pertinent to my dissertation work, however, are specifically concerned with ELS-associated alterations in the physiological systems that regulate individuals' reactivity to stress – particularly the hypothalamic-pituitary-adrenal (HPA) axis and the immune system [17, 61, 65]. These stress-reactivity systems have been particularly implicated in the pathways between ELS and psychological disorder, and are the focus of much ongoing research.

A brief overview of how the biological stress response systems function, and their adaptive significance, is provided below. Subsequently, I review the guiding theoretical model

that specifies how these stress-response systems become dysregulated in the aftermath of early-life stress and contribute to increased risk for psychological disorder.

1.4.1 Overview of the biological stress response

The biological response to acute stressors – challenges such as encountering a dangerous animal, a threatening man, or witnessing severe conflict – is mediated in a highly coordinated fashion by two distinct but interacting systems: the sympathetic-adrenomedullary (SAM) system and the HPA axis, which in turn both influence and are regulated by a third partner, the immune system. "Stress reactivity" refers to the magnitude of change in HPA, immunological, and SAM biological markers from their basal values in response to an acute psychological stressor or other environmental stimulus, a parameter that is known to vary markedly across individuals [66]. A summary of selected HPA axis, sympathetic adrenomedullary, and immune system effects during an acute stress response is provided in Table 1 [61, 67]. This coordinated stress response has been conserved across vertebrate phylogeny, and is necessary for proper physiological function and survival [68].

During the stress response, the SAM system orchestrates the immediate release of epinephrine and norepinephrine and mobilizes metabolic resources for the "fight-or-flight" response [69]. Meanwhile, on a slighter slower time scale (minutes rather than seconds), the HPA axis responds by stimulating a sharp increase in the steroid hormones called glucocorticoids via pathways running between the hypothalamus, the pituitary, and the adrenal glands. The end product of this cascade in humans is a hormone called cortisol, which is produced by the adrenal cortex. Cortisol binds to glucocorticoid receptors (GRs) in the brain and throughout the periphery, stimulating various physiological effects aimed at readying the individual to respond to the threat, as well as activating negative feedback pathways that suppress further HPA activity (see Table 1). During the "recovery" phase, once the stressor has been dealt with or disappears, cortisol levels return to their baseline values.

The immune system's acute phase response is also activated during stress, leading to increased production of certain inflammatory cytokines and proteins (e.g., interleukin (IL)-1, IL-6, IL-10, C-reactive protein, and tumor necrosis factor-α (TNF-α)). During the body's response to infection or injury, these inflammatory cytokines have a broad array of functions, including facilitating cell migration to sites of infection, signaling for cell proliferation, and initiating systemic adaptations such as fever. Importantly, those same processes are activated in the context of *psychosocial* stressors. This has been interpreted to reflect an adaptive protection mechanism, as the inflammatory response would help prevent any infection resulting from injury during combat or escape [70]. Inflammatory protein levels reach peak levels minutes or hours after the stressor, declining thereafter [67].

The HPA axis and inflammatory response are tightly interconnected. Cortisol transiently stimulates certain aspects of the immune system early on during the stress response. In a reciprocal manner, inflammatory proteins such as IL-1β activate the HPA axis to release cortisol. This systemic release of cortisol serves a vital function: cortisol binds to glucocorticoid receptors located within macrophages and monocytes, suppressing the synthesis, release, and efficacy of cytokines and other inflammatory mediators, and gradually slowing down the inflammatory process initiated during the stressful episode. Hence, cortisol is known primarily for its powerful anti-inflammatory role [71].

Table 1. Physiological stress response systems and their actions during acute stressors

HPA axis	SAM system	Immune system
1. Increased, and subsequently inhibited, glucose utilization by the brain 2. Mobilization of stored energy to the exercising muscles 3. Inhibition of reproductive physiology and behavior 4. Sharpened cognition 5. Decreased appetite 6. Transitory stimulation of immune function 7. Subsequent potent suppression of immune function	1. Secretion of epinephrine and norepinephrine 2. Dilation of blood vessels to the muscles 3. Pupil dilation 4. Increased heart rate 5. Slowing of digestion 6. Relaxation of bladder and colon 7. Stimulation of inflammatory cytokines and other immune system proteins (e.g., IL-1)	1. Increased production of cytokines and other acute-phase proteins 2. Altered lymphocyte trafficking 3. Increased production of white blood cells

These closely regulated processes indicate the adaptive significance of the stress response. Optimal levels of the SAM, HPA axis, and immune mediators are necessary to ensure that they maintain a functional balance. As described in McEwen & Seeman [72], failure to shut off the HPA axis through its negative feedback pathways can result in suppression of the immune system and a concomitant increased susceptibility to infection and disease. Alternatively, an *inadequate* response from the HPA axis would result in overactivation of the immune system and chronic inflammation, increasing risk of autoimmune and inflammatory disorders including depression [17, 72]. Likewise, repeated or prolonged exposure to the biological mediators involved in the stress response can lead to harmful wear-and-tear on the body, termed allostatic load [73].

A large body of research now indicates that the HPA axis and immune system are highly malleable during early life [17]. During development, a child's primary caregiver functions as a crucial regulator of stress responses through moderation of the child's physiological arousal levels as he or she explores the environment, and by soothing the child if it becomes over-aroused or stressed [74, 75]. Caregiver responsiveness, as well as other socio-environmental cues such as maternal stress and household conflict, are translated into altered stress-response functioning in the child [17, 61, 75, 76].

1.4.2 *Developmental programming of neuroendocrine and inflammatory biology*

The most comprehensive theoretical model describing the biological processes through which early adversity "gets under the skin" to negatively influence later health is Miller, Chen and Parker's [17] biological embedding of childhood adversity model. Their work draws heavily on concepts from several other theoretical perspectives, including life course epidemiology [1], developmental programming, and behavioral immunology [77]. Miller et al.'s model focuses largely on the role of the immune system and proinflammatory processes, but also integrates predictions about HPA axis effects that derive from previous work by other researchers.

Miller et al.'s biological embedding model posits that experiencing significant stress during childhood results in the permanent re-calibration of specific bodily systems. The immune and endocrine systems, which are highly malleable in early life, are particularly implicated: exposures during early life can have long-term effects on how these systems respond to future challenges [17, 78]. Specifically, Miller et al. hypothesize that experiencing

9

ELS leads to (1) a proinflammatory phenotype in key immune system cells, monocytes and macrophages, and (2) alterations to the endocrine system, particularly the HPA axis. Potential mechanisms through which these systems are re-calibrated include epigenetic alterations (stable changes in gene expression caused by methylation of DNA or structural changes to the chromatin), posttranslational modifications (chemical modifications to synthesized proteins), and tissue-specific remodeling. The details of these mechanisms are beyond the scope of this manuscript; readers are encouraged to refer to Miller et al. 2011 for more information and references. Lastly, Miller et al.'s model holds that these proinflammatory characteristics are exacerbated over the lifecourse as a result of concomitant behavioral proclivities such as heightened threat vigilance, poor social ties, and ineffective self-regulation, and that these systems interact to generate chronic inflammation that gives rise to chronic diseases, including depression.

Miller et al. describe the ELS-associated proinflammatory phenotype as characterized in the immune system by exaggerated inflammatory responses to acute challenge and reduced sensitivity to inhibitory hormonal signals. Monocytes and macrophages, which are the body's first-line defense against injury and infection, act as the main channel through which these altered inflammatory responses are mediated. For example, ELS-exposed individuals' monocytes will demonstrate larger *ex vivo* cytokine responses to microbial challenge, higher circulating levels of cytokines and other inflammatory proteins, and potentially disrupted adaptive immune function [17]. This combination of effects will lead to high levels of chronic inflammation throughout life.

In the endocrine system, the proinflammatory phenotype is augmented by concomitant alterations in the HPA axis' responses to challenge and diurnal output. Miller et al.'s predictions focus on diurnal output of cortisol, which they posit will be higher in ELS-exposed individuals. At the same time, the model anticipates that the immune system's macrophages may be desensitized to the effects of cortisol, leading to both elevated cortisol output and high chronic inflammation [17]. Other researchers have focused more on the HPA axis' acute response patterns, hypothesizing that early trauma will "prime" the HPA axis to subsequently react to acute stressors in an exaggerated, hyper-active manner [79].

Importantly, it has also been hypothesized that *two (or more) different patterns of atypical HPA axis reactivity* – in particular, blunted or heightened responses – may be encoded in the aftermath of ELS, depending on interactions with genetic predispositions or other factors [72, 80]. Although this hypothesis has not yet been extended to include differential patterning of inflammatory responses to psychosocial stress, the tight interconnections between the immune system and HPA axis, as well as preliminary empirical evidence (see below), suggest that this idea is plausible. These different stress-response patterns may, in turn, promote differential susceptibility to lifetime psychological disorder [81-83]. Therefore, distinct cortisol and immune system reactivity patterns may represent causal mechanisms underlying the divergent long-term mental health outcomes observed in ELS-exposed populations.

Empirical evidence for the biological embedding model and associated hypotheses stems from distinct but overlapping literatures, and is reviewed in detail below.

1.4.3 Early-life stress predicts HPA axis dysregulation

In line with the theoretical predictions described above, a range of studies in both animal models and humans have consistently reported significant alterations in cortisol reactivity associated with early-life stress exposure.

In animal models of ELS, rat pups exposed to low levels of maternal care behaviors such as licking and grooming exhibit heightened HPA axis responses to stressors, and increased behavioral signs of fearfulness, in comparison to pups of high-licking mothers (e.g., [84, 85]). These effects appear to be mediated by alterations in expression of glucocorticoid receptors in the brain and peripheral tissues, as hypothesized by Miller et al. [17] and others. Rat pups exposed to extended periods of maternal separation (and low levels of licking and grooming) have high DNA methylation levels on hippocampal GR genes, resulting in decreased GR expression and hyperreactive, prolonged stress responses [86]. As well as lifelong HPA hyperreactivity, these rats exhibit exaggerated startle responses, increased anxiety and anhedonia, and cognitive impairments [87]. In contrast, rat pups with normal parenting have reduced levels of DNA methylation on GR genes, increased hippocampal GRs and efficient HPA stress responses [88]. However, research using primate models of ELS demonstrates patterns rather different than those observed in rodents. In primates, prolonged separation stress has been associated with diminished HPA axis activation and dysregulated glucocorticoid feedback [89-91]. The explanation for these somewhat conflicting results is not clear, although the rat and primate experimental models differ substantially, and neither is a perfect corollary to the human ELS-and-cortisol reactivity findings.

Although there is evidence of HPA axis dysregulation among human ELS survivors, the pattern of the abnormalities is somewhat inconsistent across studies, and in adults it appears to differ by the psychological status of the population studied. In toddlers and children exposed to neglect or maltreatment, abnormally blunted (hyporeactive) cortisol responses to stressful challenges have been demonstrated in both short-term, acute situations as well as across a period of many days [92-95] . Other studies, using infants, have found that family adversity or high maternal stress during pregnancy was associated with unusually high cortisol reactivity (hyperreactivity) [76, 96].

In studies examining adolescents, two groups of researchers have found that youths exposed to childhood maltreatment demonstrated blunted cortisol responses to a standard psychosocial stress test that were significantly [97] and marginally significantly [98] (p=0.07) lower than those of control groups. In the first of these studies [97], youths with a history of depression were excluded, while mental health status in the second study was not assessed [98]. However, one recent case-control study of depressed and control adolescents found that combined ELS and chronic current stress predicted elevated and prolonged cortisol responses, regardless of depression [23].

In adults, the few studies examining HPA reactivity to stress among patients with current psychiatric disorder find that cortisol responses are significantly elevated compared to non-ELS controls. Heim and colleagues [99] found that women with co-morbid depression and PTSD who had been physically or sexually abused in childhood demonstrated significantly increased cortisol responses to a psychosocial stressor, compared with both depressed/non-ELS and non-depressed/non-ELS controls. Using an all-male sample, Heim et al. [100] also reported that the combined pharmacological Dex/CRH test (a measure of cortisol release after administration of a synthetic glucocorticoid) resulted in increased cortisol production among depressed ELS-exposed participants.

In contrast to findings in ELS-exposed patients with psychiatric disorder, studies in healthy adult ELS survivors with no current mood or anxiety disorders have typically found blunted HPA responses to stress. Some of these studies excluded participants with any lifetime history of depression, although two [101, 102] excluded participants only on the basis of

current or recent psychiatric disorder. Four studies [22, 81, 102, 103] found that healthy men and women with a history of ELS but no current psychiatric disorder exhibited significantly lower cortisol responses to a psychosocial stressor than did non-ELS controls. In two studies using the Dex/CRH test, healthy ELS-exposed adults also had significantly blunted cortisol responses [104, 105]. One study, however, found that cortisol reactivity among ELS-exposed adults with no psychiatric disorder was no different than that among controls [100].

Two studies have examined cortisol reactivity among ELS-exposed persons with different levels of current psychological disorder. In the first study, adult women with a history of childhood abuse and current major depression exhibited significantly higher cortisol responses compared to abused women without current depression, as well as compared to non-abused women with current depression and non-abused, non-depressed control women [99]. In the second study, the opposite effect was found: adolescents with a history of childhood maltreatment and current moderate-to-severe depression exhibited blunted cortisol stress responses, while those with a history of maltreatment but only minimal (or no) current depression symptoms exhibited higher and more prolonged cortisol responses [106].

1.4.4 Early-life stress predicts proinflammatory phenotype

Although less research has focused on the mechanisms of ELS-related programming of the inflammatory system's response to stress, many cells of the immune system contain glucocorticoid receptors, which could be modified by the same epigenetic processes as hippocampal glucocorticoids [61]. There is a wide array of empirical research investigating the evidence for a proinflammatory phenotype following ELS exposure. However, a variety of ELS definitions – some broader than that used in my dissertation work – have been utilized, and the majority of these studies focus on basal levels of inflammation rather than immune stress reactivity.

In three studies, Danese and colleagues found that prospectively evaluated maltreatment, socioeconomic disadvantage, and social isolation during childhood were associated with significantly elevated circulating inflammatory proteins (CRP and fibrinogen) at age 32 in a population-representative New Zealand sample [107-109]. Similar associations have been observed in middle-aged and older adults. In a subsample of the Midlife in the United States (MIDUS) Survey, early-life adversity predicted higher inflammation levels (IL-6, fibrinogen, endothelial leukocyte adhesion molecule-1, and soluble intercellular adhesion molecule-1) – although only among African Americans, and some of the associations were attenuated after adjustment for confounders [110]. Similarly, older adults who reported multiple childhood adversities had higher circulating levels of IL-6 and TNF-alpha compared to adults without adversities [60]. Only the Danese et al. 2008 study separately analyzed inflammation levels among depressed ELS-exposed adults and non-depressed ELS-exposed adults. The authors found that although both groups exhibited elevated inflammation, the effect was far more pronounced among depressed ELS-exposed adults [108].

Interestingly, these ELS-associated differences in inflammation levels appear to emerge quite early in life: in a later study, using a sample of United Kingdom twins, Danese and colleagues found that inflammation (CRP levels) was significantly heightened in maltreated 12-year-old children, but only if those children were also currently depressed [111]. Very early ELS may also have an effect: *in utero* exposure to high maternal chronic stress predicted higher *in vitro* stimulated production of TNF-alpha and IL-8 in infant cord blood samples [112].

There are also several reports of proinflammatory phenotypes among adults exposed to low familial socioeconomic status (SES) during childhood (more typical measures of ELS, such as abuse or neglect, were not assessed). Low childhood SES predicted higher adult concentrations of IL-6 [113], expression of transcripts bearing response elements for NF-kappa B, and other measures of genetic up-regulation of proinflammatory processes [114].

The Miller et al. [17] biological embedding model does not specifically discuss whether ELS exposure may result in the re-calibration of inflammatory responses to acute psychosocial stressors, and individual differences in these responses are not well understood. However, there is preliminary evidence that such inflammatory stress responses might play a role in the pathway between ELS and later psychological problems. Pace et al. [115] reported that both basal IL-6 as well as IL-6 and NF-κβ DNA binding responses to stress were increased in depressed males with ELS. Similarly, Carpenter et al. [116] found that childhood trauma exposure predicted greater stress-induced increases in IL-6 concentration among a small sample of adults aged 18-64 years, controlling for confounders. This study differed from the Pace study in that it excluded participants with current mood or anxiety disorders; also, no differences in baseline IL-6 were found between maltreated and control groups. In a different design, Miller et al. [117] reported that teenage females exposed to harsh familial environments displayed increasingly pronounced *in vitro* IL-6 responses to immune stimulation over a period of 1.5 years, a finding consistent with the hypothesis that early environments can continue to calibrate immune reactions over time.

No studies have examined the relationship between *in vivo* measures of cytokine and cortisol responses to stress among ELS-exposed individuals, although theoretical models predict that these responses should be related.

1.4.5 *Stress reactivity dysregulations are associated with psychological disorders*
The HPA axis and immune system are widely implicated in the etiology of mood and anxiety disorders, increasing the plausibility of a pathway between ELS-related stress reactivity dysregulation and psychological outcomes. Clinical depression is often conceptualized as a neurobiological correlate of the chronic stress response, characterized by dysregulation of the HPA axis [118, 119]. More recently, it has been suggested that depression may be caused in part by pro-inflammatory cytokines. This theory, called the "inflammatory and neurodegenerative hypothesis of depression" [120, 121], posits that both internal and external stressors – including psychosocial stress – result in systemic inflammation via multiple pathways, leading to altered HPA axis functioning, increased neurodegeneration, decreased serotonin levels, and finally culminating in symptoms of depression. Both systemic inflammation and dysregulated immune responses to stress could be responsible for the observed HPA axis dysregulation in depression.

Depression and anxiety generally (in samples not subdivided by ELS status) are often associated with chronically elevated basal levels of cortisol as measured in saliva, urine and blood [122]. Such hyperactivation of the HPA axis is believed to result, in part, from reduced feedback inhibition by endogenous glucocorticoids, which can happen through a multitude of pathways [77]. Many studies have also found that depression and anxiety symptoms are associated with blunted cortisol stress reactivity [123-127]. This is consistent with considerable evidence that chronically stressed people exhibit reduced cortisol responses to acute stressors [77]. However, as noted above, depression in ELS-exposed samples has been found in preliminary studies to predict *elevated* cortisol reactivity, suggesting that either there

is no systematic relationship between ELS and cortisol reactivity in depression, or that the story is more complex than is currently realized.

Mood disorders are consistently associated with elevated levels of basal inflammation, at least in a large subset of patients [128-130]. Several lines of evidence suggest that inflammation may in fact play a causal role in the onset of major depression: First, a large variety of pro-inflammatory cytokines (e.g., IL-1, IL-6, TNF-α) are significantly elevated in depressed patients; second, many medical illnesses that are characterized by chronic inflammation are also accompanied by depression; and third, administration of proinflammatory cytokines in both humans and animals induces depressive symptoms (see [128] for a review). In contrast, the few studies that have investigated inflammatory *stress responses* in depressed individuals have found conflicting results. The first such study found no reliable difference in stress-induced cytokine increases between MDD patients and controls, although the post-stressor recovery period was probably not long enough to detect an effect [131]. A second study found that markers of inflammation (IL-6, TNF-α, and CRP) showed a more pronounced stress-induced increase among depressed patients compared to healthy controls [132] (Weinstein et al. 2010), while a third study found that women with high levels of subclinical depression symptoms showed a *reduced* stress-induced increase in circulating IL-6 compared to controls [133]. The reason for these conflicting results is not clear, but all of these studies had small sample sizes and require replication in larger samples. Exposure to ELS was not assessed in any of these studies, and it is possible that a history of ELS could modify the association between depression and inflammatory responses to stress.

Animal models have provided limited indication that early-life environments activate proinflammatory phenotypes that can lead to depressive-like behaviors. For example, elevated gene expression of TNF-alpha has been demonstrated in guinea pig pups separated from their mothers, and administration of anti-inflammatory compounds blocked the depression-like passive behaviors (e.g., crouched stance, closed eyes) seen in separated pups [134]. Several studies in rats have showed that psychological stress increases basal levels of pro-inflammatory cytokines, and one study [135] linked this inflammation to subsequent depression symptoms [136-139]. However, these stressors were administered in adulthood, leaving the results' comparability with the effects of ELS unclear. Very little work has been done in non-human primates. As described above, most of the evidence about the inflammatory sequelae of ELS emerges from human studies.

1.5 Methodological Issues in the Early-Life Stress and Psychobiology Literature

The available scientific literature has failed to converge on the hypothesis that early-life stress increases risk for mood and anxiety disorders via permanent alterations to neuroendocrine and immune systems. Although there is solid evidence that experiencing early-life stress increases risk for mood/anxiety disorder, the evidence remains tenuous that (a) ELS induces permanent changes in the neuroendocrine and immune systems, and (b) that such biological changes are responsible for the increased incidence of depression/anxiety among ELS-exposed persons. Methodological limitations and conflicting findings in existing studies have so far prevented clear inference about the nature of this relationship. The key issues are outlined below.

First, evidence regarding *patterns* of stress reactivity among ELS survivors – particularly cortisol reactivity, for which there is more data – is notably divergent, with indications that

reactivity phenotype and psychological status covary. Specifically, many studies have found that ELS-exposed persons with no current psychopathology exhibit blunted cortisol responses to acute stress, while the few studies examining ELS-exposed persons with current major depression report that they exhibit exaggerated responses to stress. These results contrast with what has been found generally in depressed patients, who typically exhibit blunted cortisol responses to stress and increased basal inflammation. Further confusing the issue, one rigorous study found blunted cortisol stress responses in depressed ELS-exposed persons and heightened reactivity in non-depressed ELS survivors [106]. The literature on ELS and immune stress reactivity is currently too small to draw any conclusions, although the literature on ELS and *basal* inflammation fairly consistently finds a positive relationship.

While the psychobiology of early-life stress and mood/anxiety disorders is complex, it is possible that methodological limitations of previous studies in this field have biased their conclusions. Nearly all previous studies examining the interplay between ELS, depression/anxiety, and stress reactivity use case-control or cross-sectional designs. These studies' samples are usually restricted such that participants are either currently psychologically healthy or have a current mood disorder. In both situations, current depression symptoms are treated as a statistical confounder. Participants' long-term histories of psychological problems are generally left unexamined, even though – as described in detail above – mood and anxiety disorders often manifest in unusually chronic and severe forms in ELS-exposed populations. This design may have resulted in the misclassification of study participants with respect to their long-term experiences with psychological disorder, which is the outcome of greatest interest to researchers and policymakers.

Age of onset for mood disorders is typically between 20 and 30 years in ELS-exposed persons [4, 9], but many previous studies have used samples of adolescents (<18 years) [23, 97, 98, 106, 117] or young adults (<30 years) [102, 103, 115]. This sampling strategy may lead to misclassification due to right-censoring, as the participants' future lifetime psychological experience is unclear. While examining the relationship between early-life stress and biological stress reactivity in young populations has many advantages (including the ability to assess stress reactivity patterns in those with early-onset psychological disorder at a crucial period of development), such misclassification may lead to incorrect inferences about the stress-reactivity patterns associated with – or even causing – recurrent mood and anxiety problems.

Furthermore, although many studies restricted their samples to only include ELS-exposed individuals who were psychologically healthy (or, alternatively, had current clinical depression), those "healthy" ELS-exposed groups very often had significantly higher levels of depression symptomatology when compared to the control groups. These psychologically "healthy" ELS-exposed samples could therefore have included people who, while not meeting clinical criteria for depression, may still have had subclinical disorder. By not stratifying further on depression symptoms *among* the ELS-exposed groups, previous analyses may have missed additional psychobiological heterogeneity that existed within these groups.

Investigations of this relationship in older populations that can incorporate measures of their *long-term,* recurrent psychological problems are needed. Furthermore, investigations of whether baseline inflammation levels or inflammatory responses to acute stress differ between ELS-exposed individuals with and without a long-term history of psychological distress would be helpful. Targeted investigations that explore population variability in mood/anxiety symptoms and any concomitant biological differences among ELS-exposed persons would aid

in the effort to establish whether these biological pathways are causal mechanisms. Likewise, it is critical that this field incorporate analyses further exploring the patterns and covariance of immune system and cortisol stress reactivity in ELS-exposed individuals. As detailed above, stress reactivity is a physiologically complex process, and a broad examination of its multiple interacting component parts is key to understanding its role in explaining the link between early-life stress and psychological disorder.

1.6 Motivation for the Current Research Project

Research on this topic, while not a traditional focus in the field of epidemiology, has important population health implications. Both early-life adversity and mood/anxiety disorder are critical public health problems, with broad segments of human populations worldwide negatively affected by one or both. Establishing the biological pathways that underlie the association between ELS and mood/anxiety disorder could (1) lead to potential new behavioral and/or pharmacologic treatments, (2) indicate key biomarkers to use as interim outcomes in intervention programs, (3) provide a new target for promoting the development of resilience in at-risk populations, and (4) serve to reinforce the message that traumatic early-life experiences are detrimental to the health of the population and should be a cause for concern among policy-makers. Additionally, as much of the existing research in this area has been conducted by psychologists and biologists who are typically not trained in rigorous research methods, contributions by epidemiologists – with an eye towards reducing bias, addressing issues of confounding, and increasing the generalizability of results – will be valuable.

It was these goals that motivated the current research. My dissertation project uses a large sample of British white-collar workers to explore the longitudinal relationship between early-life stress, symptoms of depression/anxiety and their joint effect on a comprehensive measure of hormonal and immunological stress reactivity in later life. This project aims to address several of the shortcomings of previous studies, and advance our understanding of how early-life adversity may program distinct lifetime trajectories of psychological vulnerability and resilience through key biological pathways. My Aims were as follows:

1. Determine whether cortisol stress reactivity patterns observed in the ELS-exposed adults differ from those observed in non-ELS-exposed adults, and whether the patterns are differential according to their longitudinal history of psychological distress.

2. Identify whether distinct clusters of stress-reactivity patterning exist within cortisol and a key inflammatory protein (IL-6), and if so, whether the clustering pattern within one biomarker is associated with the clustering in the other.

3. If such clusters are identifiable, determine whether self-reported early-life stress, longitudinal history of psychological distress, and other theoretically important variables predict individuals' membership in those clusters.

Chapter 2: Study Design and Methods

2.0 Study Sample

The data for this analysis are taken from the Heart Scan Study (Principal Investigator: Andrew Steptoe, University College London), a psychophysiological investigation of a subsample of the ongoing Whitehall II epidemiologic cohort. The Whitehall subsample which comprised the Heart Scan Study (HSS) was 543 healthy older adults selected in 2008 with the primary aim of investigating the association between physiological reactivity to an experimental stressor and sub-clinical coronary artery calcification. Extensive information on the Whitehall II cohort has been published previously (e.g., [28, 140, 141]); a brief overview of the cohort and further details on the Heart Scan Study are provided below.

2.0.1 Whitehall II Cohort

The Whitehall II epidemiologic cohort was set up between 1985 and 1988 for the purpose of investigating the causes of the social gradient in health. The target population for the cohort was male and female civil servants who were between the ages of 35-55 in 1985 and working in the London offices of twenty civil service departments. Participation was requested by letter and the overall final response rate was 73%, although this varied somewhat by civil service employment grade. (Employment grades were ranked according to salary and correspond to jobs ranging from high-level administration to office clerical support.) The original sample size was 10,314 participants, approximately 67% of whom were male.

The cohort has subsequently been followed for more than twenty years, with data collections approximately every two to three years. Cohort members are invited to come to the research clinic for health screenings every five years, and questionnaires are mailed to participants between each clinic visit. Follow-up for mortality is conducted through the National Health Service Central Registry. A large variety of measures are collected on cohort members during each data wave, with particular emphasis on psychosocial variables, health behaviors, self-reported mental health and quality of life, and health outcomes. Attrition over the course of the study has been relatively low, with 67% of the original cohort followed up in 2004, the last year for which comprehensive participation data are available (this number includes those who died prior to the 2004 data collection).

Although the Whitehall II cohort cannot be considered representative of the general English population – most importantly, it is a healthy working sample that does not include manual workers or other very-low-socioeconomic status individuals, and includes relatively few women and people from ethnic minority groups – the study has many notable strengths. The cohort is extremely well characterized with respect to socioeconomic status and is reasonably representative of white-collar workers at the time of the cohort's formation. Information on an wide variety of variables has been collected over the 20+ years of the study, making it an invaluable resource for longitudinal, interdisciplinary investigations into the psychosocial determinants of health.

2.0.2 Heart Scan Study

The Heart Scan Study, which used a subsample of the Whitehall II cohort members who responded to the 2006 Whitehall questionnaire, was primarily designed to examine

whether psychobiological stress responses predict current and future coronary atherosclerosis. The study included two visits, the first of which took place during 2007-2008 and consisted of undergoing a laboratory-based experimental stressor as well as a subsequent screening for coronary artery calcification (CAC) levels. The second visit took place three years later (in 2011) and consisted solely of a secondary CAC screening. Note that the current analysis uses Heart Scan Study data from the first visit only, as I did not have access to the 2011 follow-up data. After the HSS was completed, the data were linked to six waves of the HSS participants' Whitehall II study data (from data collection phases in 1985-88, 1989-90, 1991-93, 1997-99, 2001, and 2003-2004), using their Whitehall identification numbers. Figure 1 depicts the overall design of my analysis, including the year(s), study wave, and mean participant age when each key variable was collected.
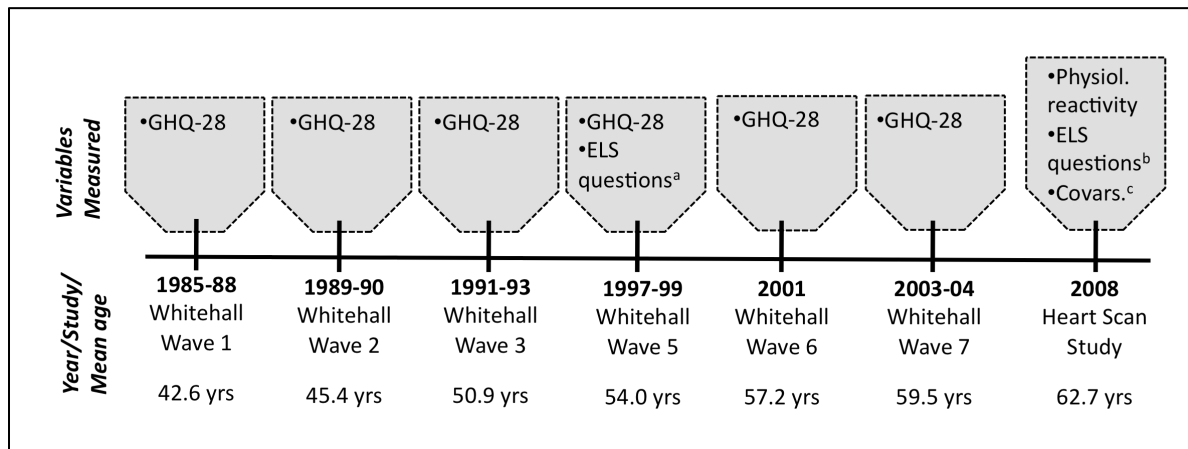

Figure 1. Overall design of the current analysis. Data collected during Whitehall II waves 1-3 and 5-7 were available for all Heart Scan Study (HSS) participants. Timeline shows year(s) of each data collection and mean age of HSS participants at each collection. Gray boxes list key variables collected during each study wave that were used in the present analysis: [a]Physical abuse, parental divorce, parental conflict, parenting style, separation from mother/time spent in orphanage; [b]Early-life stress (ELS) questions: age at death of parents, family mental illness/substance abuse; [c]Covariates: BMI, smoking status, last Whitehall employment grade, CESD score, age, gender, time of laboratory visit. GHQ-28, General Health Questionnaire-28 item version.

The physiological stress reactivity parameters collected during the HSS included cortisol and cardiovascular and inflammatory protein markers. This analysis focuses only on the cortisol and certain inflammatory measures. Questions on a range of early-life experiences, health behaviors, sociodemographic variables, and other health indices – included in either the HSS protocol or in previous Whitehall waves – were used for the current analysis (the various outcome, predictor, and covariate measures are described in detail below).

As the HSS was designed to examine whether psychobiological stress responses predict coronary atherosclerosis, it was necessary to avoid enrolling individuals with any disease state that might affect both their physiological stress reactivity and atherosclerosis risk, thus leading to a spurious association between stress reactivity and atherosclerosis. Potential HSS participants were excluded if they were currently taking anti-inflammatory medications or reported a serious illness within the past five years (n=228). As major depression has been associated with altered stress responses [123] and cardiovascular disease [142-144], the HSS investigators also opted to exclude individuals who had been diagnosed with major depression

within the previous five years, or were *currently* taking anti-depressants or anti-psychotics (n=18). This information was collected from Whitehall II study members during screening phone calls for participation in the HSS (i.e., in 2008), and was verified from data collected in previous phases of the main Whitehall II study. Psychiatric diagnoses prior to 2003 and/or usage of psychotropic medication prior to 2008 were allowed, as were psychological problems that remained undiagnosed.

Mean age of the final HSS sample, in 2008, was 62.7 years (range: 54-76); 55.2% were male; all were of white European origin. Although this is obviously an older-adult sample, 56.5% of the participants were still in full-time employment. Selection of participants was stratified by Whitehall grade of employment (current or most recent) to include higher-, middle-, and lower-socioeconomic status participants. Participants from lower employment grades were more likely to decline to take part when compared with those from higher grades (38.6% vs. 20.3%, respectively).

The youngest woman in the HSS was aged 55 years, and all women reported postmenopausal status. Female HSS participants were not excluded if they were taking hormone replacement therapy. A small percentage of the female participants (7.5%; n=41) reported taking hormone replacement therapy in Whitehall wave 7 (2003-04), but the investigators did not collect additional information on HRT in 2008.

The final analytic sample for the current analysis included all HSS participants who responded to questions regarding their early-life stress exposure (n=424). The Heart Scan Study was approved by the Research Ethics Committee for University College London/UCL Hospitals, and all participants provided written informed consent. The current investigation was a secondary analysis of de-identified data and the **Committee for Protection of Human Subjects at the University of California, Berkeley waived the requirement for formal review of this research.**

2.1 Definition of Key Study Variables

2.1.1 *Exposure variables*

Early-life stress variable. To create the composite variable delineating exposure to early-life stress, I used questions taken either from the HSS or from Whitehall II wave 5 (1997-1999). Possible reported ELS categories (all occurring before age 16) included physical abuse, separation from mother or time spent in an orphanage for one or more years, parental death, serious familial mental illness or substance abuse, parental divorce, frequent parental conflict, and harsh parenting style. As such, ELS as defined in this analysis includes categories of general adversity [22, 23], and is not limited to childhood maltreatment.

Death of a parent was assessed in the HSS questionnaire using the following text: "Up until the age of 15, did anyone in your household die, and if so, who?" Written responses were visually inspected and participants were coded as having experienced the death of a parent if they had written in "mother" or "father" or both. All other ELS questions, except those on parenting style (see below) used binary yes/no responses, and are reproduced in Table 2 below.

> **Table 2. Selected early life stress questions**
>
> *Up until the age of 15, did any of the following things happen:*
>
> 1. Did anyone in your household die, and if so, who?
> 2. Did anyone in your household have a serious problem with alcohol or drugs?
> 3. Did anyone in your household become seriously depressed or attempt suicide?
> 4. Did your parents get divorced?
> 5. Did your parents very often argue or fight?
> 6. You were ever separated from your mother or in an orphanage for a year or more.
> 7. You were physically abused by someone close to you.

Responses to parenting style questions, which were drawn from the MIDUS study [145, 146] and are reproduced in Table 3 below, were based on a 4-point Likert scale. Higher scores corresponded to higher parental harshness and strictness, and lower affection, understanding, confiding, attention, and expectations. Following the example of Stansfeld and colleagues [147], participants whose parenting style scores were in the top tertile (score ≥14; range: 0-21) for either their mother or father were classified as having a "harsh parent."

> **Table 3. Parenting style questions**
>
> *Please show how you remember your mother/father during the years you were growing up:*
> 1. How much did she/he understand your problems and worries?
> 2. How much could you confide in her/him about things that were bothering you?
> 3. How much love and affection did she/he give you?
> 4. How much time and attention did she/he give you when you needed it?
> 5. How strict was she with her/his rules for you?
> 6. How harsh was she when she/he punished you?
> 7. How much did she/he expect you to do your best in everything you did?

The final ELS exposure variable was summed across ELS categories and then dichotomized into any ELS (ELS+) vs. none (ELS−). This categorization method was chosen on the basis of evidence that even exposure to only one type of early-life adversity can substantially increase risk for psychological problems (e.g., [6, 40]), and because the data did not include measures of certain categories of ELS exposure (e.g., sexual abuse, severe neglect) that frequently co-occur with other categories [4, 39-41]. (See Introduction for more details on the co-occurrence of ELS categories.)

Psychological distress variable. Participants' psychological distress symptoms were measured at each Whitehall wave using the General Health Questionnaire-28 (GHQ-28), when participants' mean ages were 42.6, 45.4, 50.9, 54.0, 57.2, and 59.5 years, respectively. Mean duration of time between the baseline and final GHQ-28 assessments was 17 years (range: 15-19 years). The GHQ-28 is a widely used and validated Likert-scale instrument that identifies individuals with an increased likelihood of current psychiatric disorder, particularly depression and anxiety [148, 149]. Sensitivity and specificity of the GHQ-28 in the Whitehall II sample are approximately 73% and 78% [150] compared to the Clinical Interview Survey, which uses "case criteria" to determine probable psychiatric cases vs. non-cases [151]. A cutoff score of ≥5 indicates presence of significant psychological distress. Participants who never scored ≥5 at any of the 6 Whitehall waves were classified as having no history of psychological distress in adulthood ("no distress"); those with a ≥5 score at 1 or 2 waves were classified as having a

minimal history of adulthood psychological distress ("minimal distress"); and those with a ≥5 score at 3 or more waves were classified as having a history of recurrent psychological distress in adulthood ("recurrent distress"). Finally, participants were classified into one of six combined ELS/distress indicator groups: non-ELS/non-distress, non-ELS/minimal-distress, non-ELS/recurrent-distress; ELS/non-distress, ELS/minimal-distress, and ELS/recurrent-distress.

2.1.2 Outcome variables

HSS laboratory session protocol. All physiological reactivity data were collected during a controlled, laboratory-based stress-inducing protocol. Laboratory visits for the Heart Scan Study took place in the morning (beginning 9:15 am) or afternoon (1:00 pm). Participants were instructed to refrain from vigorous exercise and alcohol the evening prior to the laboratory visit, and from smoking or drinking caffeine for two hours prior. Upon arrival, participants provided written informed consent and were allowed to rest for 30 minutes, after which they underwent the stress reactivity tasks.

The stress task protocol consisted of two 5-minute cognitive-behavioral challenges designed to elicit mental stress. Order of the challenges was randomized. The first challenge was the Stroop test, a computerized color-word interference task where a color word (e.g., "yellow") is presented on the screen in an incongruent color. Participants are instructed to choose a word matching the printed font color. The program indicates whether the selection was correct, and continues to a new set of words. Presentation speed was programmed to vary in response to performance accuracy to maintain pressure. The second challenge was mirror tracing, involving the tracing of a star with a metal stylus using only a mirror image. Errors are registered with a loud beep emitted by the apparatus (Lafayette Instruments Corp., Lafayette, IN, U.S.A.). A laboratory assistant timed each task and recorded the number of errors to augment the psychological pressure. Both tasks have been used in similar studies examining the physiological effects of acute stress, and reliably elicit HPA axis reactivity (e.g., [152, 153]). Immediately after each task, participants rated its perceived stressfulness using Likert scales; scores were averaged to create an overall task-stressfulness rating.

Cortisol. Salivary cortisol, measured five times over the 85-minute laboratory session, was used to assess HPA responses [154]. After the resting period, a baseline saliva sample was taken. Samples were then taken immediately after the stress tasks (~10 min after the baseline sample) and at 20, 45, and 75 min post-task. Samples were obtained using Salivette cotton roll collection devices (Sarstedt, Rommelsdorf, Germany). Samples were stored immediately at -30°C until assaying. Cortisol levels were assessed at the University of Dresden using a time-resolved immunoassay with fluorescence detection. Intra- and inter-assay coefficients of variation were less than 8%.

Interleukin (IL)-6. IL-6 is a proinflammatory cytokine that is stimulated during acute psychosocial stress via activation of the sympathetic nervous system [155]. Plasma IL-6 was assessed through four whole blood samples taken during the HSS laboratory session: at baseline, immediately post-task, 45 min post-task, and 75 min post-task. Samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA), a blood coagulant. Samples were immediately centrifuged at room temperature for 10 min at 3000g. Plasma aliquots were stored in polypropylene tubes and frozen at -80°C until analyzed. Plasma IL-6 concentration (in pg/mL) was measured by an enzyme-linked immunosorbent assay. Values

lower than the detection limit (0.08 pg/ml) were assigned values of half the detection limit. The coefficient of variation was below 10%.

2.1.3 Covariates

A conceptual model of the hypothesized associations among my study variables is depicted in Figure 2. Age and gender are potential confounders of the relationship between ELS/psychological distress status and physiological stress reactivity. Such confounding could occur if, for example, older participants were less likely than younger participants to have experienced ELS and/or psychological distress, and also had consistently different cortisol responses compared to younger participants. Both of these particular associations have been documented in other samples [6, 105, 156, 157]. To account for the effects of this possible confounding, I statistically controlled for these variables in all analyses.

The timing (morning vs. afternoon) of participants' laboratory visits, although unlikely to be correlated with ELS/distress status, does affect basal cortisol levels and potentially basal inflammatory protein levels, as both cortisol and IL-6 exhibit pronounced circadian rhythms [61, 158]. As the majority of studies in this field adjusts for time of stress testing to ensure full control of any data-based confounding, I also statistically controlled for time of testing.

Participants' most recent Whitehall employment grade (as a measure of their social class), body mass index (BMI) and smoking status (current smoker yes/no) were possible mediating variables in the relationship between ELS, psychological distress symptoms, and physiological reactivity [159, 160]. The *total effect* of ELS on psychological distress is the total association between the exposure and outcome variables, regardless of the mediating pathway. Social class, BMI, and smoking status make up other potential pathways through which ELS increases risk for psychological distress, and they in turn affect physiological reactivity; their combined effect is referred to as the *indirect effect* because it is not the mediating pathway of interest. In contrast, the *direct effect* is the association between the exposure and outcome variables that operates solely through the mediating pathways of interest. Because I wanted to identify, as closely as possible, the *direct effect* of ELS/distress status on physiological reactivity, I statistically controlled for employment grade, BMI, and smoking status.
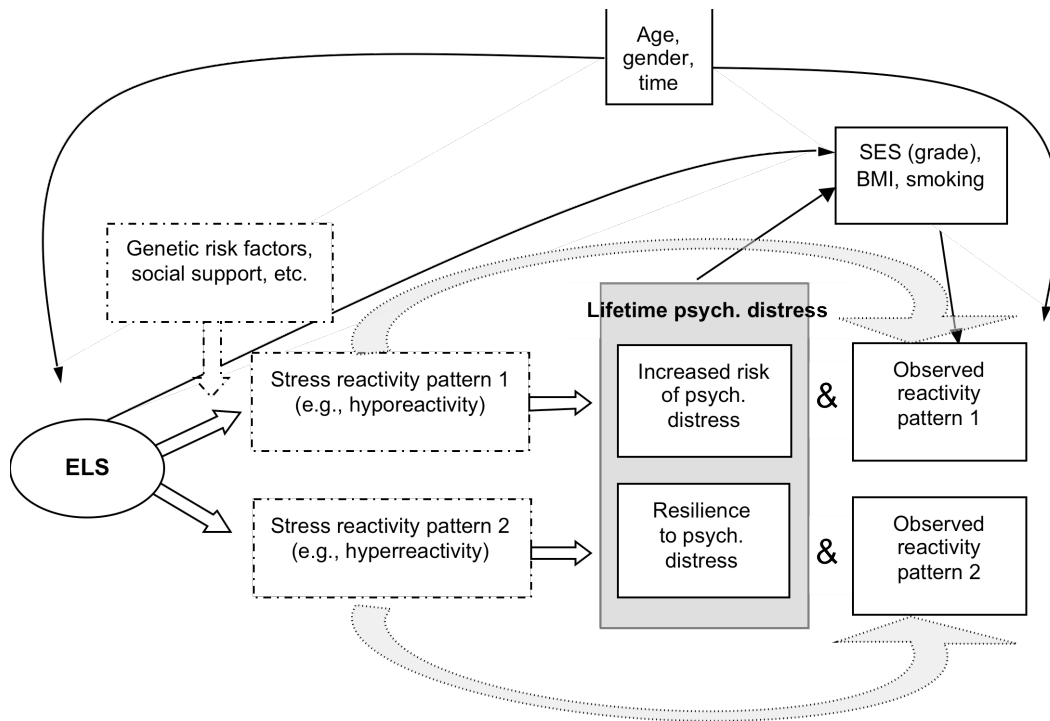
Figure 2. Conceptual model for the analysis. Solid-outline figures (e.g., "Age, gender time") denote variables observed in the Whitehall or HSS. Dashed-outline figures (e.g., "Genetic risk factors" and "Stress reactivity pattern 1") denote unobserved variables.

2.2   Statistical Analysis

Statistical analyses were conducted using Stata version 11.0 (Aim 1 analyses; StataCorp, College Station, Texas) or SAS version 9.2 (Aims 2 and 3 analyses; SAS Institute, Cary, NC).

Univariable analyses. I first examined the univariable frequencies, means, and standard deviations (SD) of the individual and composite early-life stress variables, the six psychological distress symptoms variables, and the biomarker outcome variables (cortisol and IL-6). I plotted histograms and box plots to examine the distribution of each of these variables and assess the presence of outliers, and in the case of the outcome variables, deviations from normality and skewness.

Bivariable analyses. After forming the composite ELS/distress groups, I tested for group differences in sociodemographic and physiological characteristics (age, gender, SES, BMI, smoking status) using analysis of variance for continuous variables and chi-square analyses for categorical variables.

2.2.1   *Aim 1: Cortisol stress reactivity*

Overall approach. For my first Aim, the overall goal was to determine whether cortisol stress reactivity patterns observed in ELS- and psychological distress-exposed adults differed from those observed in ELS-exposed adults who were *not* exposed to psychological distress, and in turn whether both of these groups' cortisol reactivity trajectories differed from *non-ELS-exposed* adults'. The data used for this analysis had a hierarchical structure, with cortisol measurements over time (level 1) nested within individuals (level 2). Therefore, the analysis

23

technique selected had to account for this dependence within each individual's cortisol measurements, which would otherwise violate the independence assumptions of traditional regression models [161]. If this violation of independence is not accounted for, the estimated magnitude of the predictor variables' standard errors can be wrong – in particular, the SEs of the time-independent predictor variables is likely to be underestimated, and the SEs of the time-dependent predictors overestimated [162].

In the field of psychoneuroendocrinology, statistical analyses of repeated-measures biomarker data (such as this project) are typically limited to repeated-measures analysis of variance (rmANOVA) and/or area-under-the-curve (AUC) calculations. However, these methods have a number of limitations. Repeated-measures ANOVA does not allow for incomplete participant outcome data – i.e., participants who are missing just one of several repeated biomarker measures are completely dropped from the analysis – and the measurements must be equally spaced in time, which is often not possible or desirable in the overall study design. AUC analyses, while providing a valuable index of overall biomarker output over a given period of time, cannot provide information on certain theoretically interesting parameters, such as the *slopes* of participants' biomarker responses to, or recoveries from, an experimentally induced stressor.

Growth-curve modeling. Due to these limitations, I instead used piecewise multilevel growth-curve modeling (GCM) to describe participants' cortisol response trajectories, and to assess whether combined ELS/distress history explained any of the variance in these trajectories after controlling for covariates. Growth-curve modeling is a form of random coefficient analysis, in which regression coefficients (the intercepts and slopes) are allowed to vary randomly between subjects. Although random coefficient analysis can be a flexible and powerful tool, it is important to note that inherent in the analysis technique are untestable assumptions that the random regression coefficients have a bivariate normal distribution with mean zero and unstructured covariance matrices.

Like other methods used to analyze repeated-measures data, GCM can handle multiple independent predictors and data that violate assumptions of independence. However, GCM also offers certain additional unique advantages: it allows for incomplete participant data and occasions of measurement that are unequally spaced in time [163]. Although its use in modeling repeated-measures biomarker data is still relatively novel, it has been used in similar previous studies [97, 164, 165]. The piecewise approach allowed me to separately examine three theoretically interesting aspects of the cortisol response trajectories: baseline values, slope of the cortisol reaction to the stressor, and cortisol recovery from the stressor [166]. Coding for the growth-curve modeling was derived from the Rabe-Hesketh & Skrondal text [167] and Llabre et al. [168].

All statistical analyses used the natural logarithm of cortisol to correct for skewness; however, for ease of interpretation, raw data are displayed in figures. I plotted the mean observed cortisol values for each ELS/distress group as a function of the five sample times (see Figure 5). Visual examination of the plotted data suggested a linear function for the reactivity period (baseline to immediately post-task) and a combined linear and quadratic function for the recovery period (immediately post-task to 75 min post-task).

Upon visual inspection and subsequent statistical analysis, cortisol trajectories for the three non-ELS groups were determined to be nearly identical, with all ps>0.46 for mean differences in cortisol at each sample time (F-test (1, 418)) between these three groups. Accordingly, I grouped these participants into one ("non-ELS") category for the piecewise

growth curve modeling. Likewise, log cortisol trajectories for the ELS/no-distress and ELS/minimal-distress groups were statistically identical, with all $ps>0.58$ for mean differences between these groups at each sample time (F-test (1, 419)). I therefore combined these two groups into a single ("ELS/low-distress") category for growth curve modeling, although their descriptive statistics and graphed cortisol trajectories are presented in the Results separately. Grouping the participants in this way did not affect inference from my results.

Model 1 examined baseline cortisol levels and growth trajectories over the reactivity and recovery periods separately, controlling for time of laboratory visit. Model 2 added fixed effects for each of the ELS/distress groups and their interactions with the time vectors (reactivity and recovery), which allowed me to test for group differences in both baseline levels and slopes during the two time periods. Model 3 added individual-level covariates (see paragraph below). Random effects were specified for the overall intercept and for slope coefficients corresponding to each ELS/distress group x time-vector interaction. The reduced form (i.e., levels 1 and 2 combined) of model 3 is specified as follows:

$$\text{Log(cortisol)}_{ij} = \beta_0 + \beta_1(\text{reactivity}) + \beta_2(\text{recovery}) + \beta_3(\text{recovery}^2) + \beta_4(\text{group 1}) + \beta_5(\text{group 2}) + \beta_6(\text{group 1*reactivity}) + \beta_7(\text{group 1*recovery}) + \beta_8(\text{group 1*recovery}^2) + \beta_9(\text{group 2*reactivity}) + \beta_{10}(\text{group 2*recovery}) + \beta_{11}(\text{group 2*recovery}^2) + \beta_{12\text{-}16}(\text{covariates}) + \varsigma_{1j}(\text{intercept}) + \varsigma_{2j}(\text{reactivity}) + \varsigma_{3j}(\text{recovery}) + \varsigma_{4j}(\text{recovery}^2) + \varepsilon_{ij}$$

where β_0 represents the intercept; $\beta_1, \ldots, \beta_3$ indicate level-2 fixed slope effects for the growth trajectories in cortisol during reactivity and recovery periods; and β_4 and β_5 represent level-2 fixed effects for group status (group 1 = ELS/low-distress; group 2 = ELS/recurrent-distress). Variables β_6-β_{11} represent fixed effects for the cross-level interactions between group and the time vector slopes, and β_{12}-β_{16} represent fixed effects for specified covariates (see section 2.1.3 above). Variables ς_{1j} - ς_{4j} represent the random slope coefficients, and ε_{ij} the occasion-specific error (level 1 residuals).

To increase the robustness of the models' inference, and to attempt to account for the strong assumptions inherent in the technique, I performed a nonparametric clustered bootstrap analysis with replacement, where participants were the clustering variable (n=50 repetitions). All reported SEs are from the bootstrap analysis. Likelihood ratio tests were performed to assess model fit. I also used empirical Bayes predictions of the level-1 residuals (ε_{ij}) for Model 3 as a further test of whether the normality assumptions for these random variables were satisfied [167].

Lastly, I also used area-under-the-curve analysis (AUC$_{\text{ground}}$) [169] to examine total output of (unlogged) cortisol over the laboratory session. These analyses controlled for all of the covariates used in the piecewise regression Model 3. While AUC analyses are subject to the limitations described above, they are useful for providing an overall indication of how much cortisol was produced by each group of interest.

Sensitivity analyses. I also conducted certain sensitivity analyses to examine covariates that, while I did not consider there to be strong *a priori* theory about their role in my conceptual model, may still be of interest. I serially added the covariates *hormone replacement therapy usage* (measured in 2003-04, so not necessarily current) and *depression symptomatology at the HSS* (measured by the CESD) to the piecewise regression Model 3, to estimate the robustness of the model to these variables' effects. I further examined the effect of gender as a potential effect measure modifier of the relationship between ELS/distress status

and cortisol reactivity [157]. Because testing an overall three-way ELS x distress x gender term was not possible due to the analytic complexity of the model, I instead simply stratified the models by gender.

2.2.2   Aims 2 and 3: Clustering within cortisol and immune stress reactivity

Overall approach. For my second Aim, the goals were to (a) identify whether distinct clusters of stress-reactivity patterning exist within cortisol and IL-6, a key inflammatory protein, and (b) if so, determine whether membership in a given cortisol reactivity cluster is associated with membership in a particular IL-6 reactivity cluster. My goal for Aim 3 was to examine whether exposure to early-life stress, history of psychological distress, and other key covariates predicted membership in specific cortisol and IL-6 trajectory groups.

The population distribution of IL-6 responses to acute psychosocial stressors has not been established, and it is unknown to what extent there is a common growth pattern versus several distinct stress-reactivity response trajectories. A few studies have examined the effect of acute stressors on IL-6, but their methods only allowed for examination of the mean and SD of the responses, and the results were somewhat conflicting [67, 170]. As for cortisol, the field of endocrinology long ago determined that acute psychosocial stressors tend, on average, to induce a certain cortisol response pattern [69], so this assumption is fairly robust – hence my usage of random coefficient models for my Aim 1 analysis. However, even with cortisol there is evidence that qualitatively different patterns of responsiveness can exist in a population, some more adaptive than others [72, 87, 171, 172]. It is possible that there is considerably more heterogeneity within the population cortisol and IL-6 stress responses than would be identifiable through these traditional mean-oriented methods, and furthermore it is possible that such heterogeneity clusters into natural, distinct groups that index something meaningful about joint HPA axis and immune functioning [173]. For example, one group might consist of individuals whose cortisol responses start out high and remain high, while their IL-6 stress responses remain low throughout, indicating that their cortisol levels are suppressing their body's immune response to stress. Another group may show the opposite pattern, perhaps indicating that their HPA axis is inadequately responding to stress and thereby permitting higher-than-desirable levels of inflammation.

To explore the existence of such clustering within cortisol and IL-6 stress responses in the Heart Scan Study (Aim 2), and furthermore to explore whether any identifiable clusters are predicted by theoretically important variables (Aim 3), I used the analytic technique of group-based trajectory modeling [174].

Group-based trajectory modeling. Group-based trajectory modeling (GBTM) is a specialized application of finite mixture modeling, which is generally designed to identify, within a heterogeneous population, groups of individuals who follow a similar longitudinal trajectory in an outcome variable over a set period of time [171, 175]. Finite mixture modeling is labeled as such because the model sums across a finite number of discrete, unobserved groups that compose the population [174]. These latent groups are conceived to be characterized by qualitatively different response patterns; however, the groups are not meant to considered literally different, only statistical approximations of underlying differences. As with other finite mixture models, GBT models are consistent estimators of the parameters of the model and asymptotically normally distributed [176].

Basic structure of the model. In Nagin's formulation, group-based trajectory modeling assumes that a given population is made up of a mixture of J latent trajectory groups. The unconditional likelihood function for the outcome data Y_i is an aggregation of the J conditional likelihood functions ($P^j(Y_i)$):

$$P(Y_i) = \sum_j \pi_j P^j(Y_i)$$

where:
- Y_i represents the longitudinal sequence of outcome measurements (e.g., cortisol) on an individual i over T periods;
- $P(Y_i)$ represents the probability of Y_i (i.e., the probability of observing individual i's longitudinal sequence of measurements), assuming that Y_i takes discrete values;
- $P^j(Y_i)$ is the probability of Y_i given membership in group j; and
- π_j is the probability of a given individual belonging to group j.

The model assumes that, conditional on membership in group j, the random variables, y_{it}, t = 1, 2, …, T, are independent. This assumption implies that:

$$P^j(Y_i) = \prod^T p^{jt}(y_{it})$$

where $p^{jt}(y_{it})$ is the probability density function of y_{it} given membership in group j. In other words, the density of Y_{it} given membership in group j is a function of the product of all the different densities of y_{it}. The group membership probabilities (π_j; $j = 1, …, J$) are estimated by a multinomial logit function with the form:

$$\pi_j = \frac{e^{\theta_j}}{\sum_1^J e^{\theta_j}}$$

where θ_j is normalized to 0. Descriptively, π_j represents "the probability that a randomly chosen individual follows group j's trajectory" [174]. Due to the specifications of this estimation procedure, each group's membership probabilities fall between 0 and 1, and across all J groups the values of π_j sum to 1. For example, in a three-group model, the group membership probabilities might be .10, .55, and .35, meaning that 10% of the population are likely to belong to the first group, 55% are likely to belong to the second group, and 35% are likely to belong to the third group.

For this analysis, the form of $p^{jt}(y_{it})$ – the probability distribution function of y_{it} given membership in group j – was assumed to follow the censored normal distribution as recommended by Nagin [174], since the longitudinal y_{it} data were continuous within a certain biological range. The boundaries for the censored normal distribution were set to be outside the observed ranges for the outcome variables.

A polynomial equation is used to model the relationship between time (sample time) and the outcome variable (e.g., cortisol levels). This linkage is established via a latent variable, y_{it}^{*j}, which in the current analysis represents the potential for exhibiting a certain level of the biological variable of interest (e.g., cortisol). While the polynomial relationship can go up to

the fourth order, in these analyses I assumed up to a second-order (quadratic) relationship for both cortisol and IL-6:

$$y_{it}^{*j} = \beta_0^j + \beta_1^j time_{it} + \beta_2^j time_{it}^2 + \varepsilon_{it}$$

where ε_{it} represents the error, assumed to be normally distributed with a mean of zero and a constant standard deviation σ. A separate set of parameters is estimated for each group j, meaning that the shapes of the trajectories can vary freely across the groups. Maximum likelihood is used to estimate the model parameters, using a SAS procedure (PROC TRAJ) developed by Jones, Nagin and Roeder [177]. This SAS procedure accommodates missing outcome measurements with the assumption that the missingness is completely at random.

Next, the "best" number of trajectories is determined on a maximum-likelihood basis, with change in the Bayesian Information Criterion (BIC) between models used to test the number of groups in the trajectory model [177]. This method, in which $2(\Delta BIC)$ is used as an approximation of the log of the Bayes factor, deals with the problem that the null hypothesis in the test (e.g., two groups or more than two groups) lies on the boundary of the parameter space, meaning that the normal asymptotic results do not hold [177].

Lastly, the model calculates the posterior probabilities of group membership. These values denote the probability that an individual with a specific outcome profile (e.g., a high IL-6 response) belongs to a particular trajectory group j. The posterior probability of an individual i's membership in group j is denoted $P(j \mid Y_i)$ and is calculated as follows:

$$\widehat{P}(j \mid Y_i) = \frac{\widehat{P}(Y_i \mid j)\widehat{\pi}_j}{\displaystyle\sum_j^J \widehat{P}(Y_i \mid j)\widehat{\pi}_j}$$

Thereafter, a maximum-probability assignment rule assigns individuals to the group for which their posterior membership probability is largest. For example, person 1 might belong to group 1 with a 5% probability, to group 2 with a 2% probability, and to group 3 with a 93% probability, in which case that person would be assigned to group 3.

For this analysis, I also performed bootstrapping to evaluate the robustness of my inference about the trajectory groups' parameter estimates and group membership probabilities. Although group-based trajectory modeling makes use of a first-order Taylor series expansion to approximate standard errors [178], I wished to compare the similarity between the results for both methods. Taylor series results are analytical and based on untestable assumptions, and it is unclear how much to trust the results if the assumptions are violated. I used a parametric bootstrap, which simulates data based on the best-fitting trajectory clustering model (determined through the BIC criteria described above). This is in contrast to a case-resampling bootstrap, which would resample individual participants. The parametric bootstrap technique assumes that the fitted model is true, which is necessary due to the dependency structure imposed on the data by the group membership. Using the fitted parameter estimates (i.e., the group percentages (π_j), β_0, β_1, and β_2,), I generated normal distributions around each parameter and then sampled group membership. I then used Proc Traj to simulate a new set of cortisol and IL-6 group parameter estimates and their 95% confidence intervals for each trajectory group, from the sampled component of the mixture. This process was repeated 1000 times for each biomarker, since the fitted models were different for the two biomarkers. The 95%

confidence intervals were constructed using the 2.5th and 97.5th percentiles of the bootstrap estimates' distribution.

Despite this attempt to improve on the basic group-based trajectory modeling approach, it is necessary to point out that this procedure still necessarily implies that convergence to the same "best" model form would be deterministic in repeated samples. This assumption would almost certainly not hold. Therefore, even the inference obtained through the parametric bootstrapping has very little meaning, since the parameter of interest would change with every new sample. The only parameter of interest that could be considered well-defined in this context is the entire probability density of Y_{it}. However, since the density function is data-adaptive, it will go to an infinite number of mixtures as the sample size gets larger. In the current study, the sample size is small enough that the variance-bias tradeoff converges on some amount of bias in return for reduced variance. Hence, my analyses for Aims 2 and 3 must be considered extremely exploratory, with only very informal inference.

In a preliminary attempt to examine the stability of the results from this fundamentally flawed parametric bootstrap procedure, I performed a further sensitivity analysis, using just the cortisol data. In this analysis, I repeated the parametric bootstrap, but conducted the full model selection procedure on 10 of the replicate datasets. In other words, instead of assuming that the model solution was known (an assumption I made in the first parametric bootstrap, since I only used the original "best" trajectory model solution arrived at earlier through the BIC criterion), I evaluated the fit of several different finite mixture models for each of the 10 replicate datasets.

Associations between biomarker trajectory group memberships. For the second part of Aim 2, my goal was to determine whether membership in a given cortisol trajectory group was associated with membership in a particular IL-6 trajectory group. As discussed in the Introduction, the actions of cortisol and IL-6 are known to be closely intertwined during acute stress responses. However, these biomarkers are rarely measured together in experimental psychosocial stressor settings, so examining their joint stress-reactivity trajectories could provide valuable insight into the complex interactions between the neuroendocrine, immune, and psychological systems, as well as whether those systems' functioning is predicted by early-life stress and other variables.

Individuals' cortisol and IL-6 levels changed simultaneously over the Heart Scan Study laboratory sessions. Therefore, for this part of the analysis, I used Nagin's dual-trajectory analysis technique, which is an extension of general group-based trajectory modeling and allows for the "joint estimation of trajectory models for two distinct but theoretically related measurement series" [176]. Unlike other statistical techniques for measuring the co-occurrence or sequential correlation of two outcome variables, which can generally only make use of one or two time points, dual trajectory analysis uses data from multiple observation points to examine the dynamic relationship between two outcomes as they unfold over time. It is thus uniquely suited to my analysis goal.

For dual-trajectory analysis, the main assumption of the single-trajectory model (i.e., conditional independence given group membership) still holds. For my analysis, I used the general form of the dual-trajectory model, which assumes that the J trajectory groups for one longitudinal outcome series (Y_1) are probabilistically linked with K trajectory groups for the second outcome series (Y_2). (This is in contrast to each Y_1 trajectory group being uniquely linked to one Y_2 trajectory group.) Again, model parameters for both sets of outcomes are estimated with a maximum likelihood estimator. As recommended by Nagin and Tremblay [176], the final dual trajectory model is estimated using the optimal single-trajectory models

(i.e., number and shape of the groups) established previously for each outcome. The results of the dual-trajectory model provide the probability of membership in each outcome trajectory group and the probabilities linking membership in trajectory groups across both outcomes [174].

        <u>Predicting group membership from covariates.</u> For Aim 3, my goal was to examine whether exposure to early-life stress, history of psychological distress, and other key covariates predicted membership in specific cortisol and IL-6 trajectory groups. Single trajectory modeling was used to answer this question, through estimation of the relationship between the specified individual-level predictor variables and probabilities of group membership in each biomarker. (See Nagin [174], Chapter 6, for a detailed explanation of the likelihood function and multinomial logit function used for this estimation procedure.) My predictor variables of interest for this analysis were the same as those used in the random coefficient growth-curve models of Aim 1. Therefore, the model included ELS/distress (using the indicator variables specified in Section 2.1.1), gender, age, employment grade, smoking status, BMI, and time of laboratory session.

Chapter 3: Results

3.0 Descriptive Statistics

Univariable analyses. The average age of Heart Scan Study participants was 62.9 years (SD: 5.67), and 54.1% (n=294) were male. Nearly 65% reported being still married, while 18.7% were single and 16.7% divorced, separated, or widowed. Only 38% (n=206) were currently in paid employment at the time of the laboratory session. Forty percent were at a high pay grade as of their most recent civil service employment, another 40% were at a medium civil service grade, and 20% were at a low grade. The mean BMI score was 25.9 (SD: 0.17), indicating that most HSS participants were somewhat overweight. Only 30 participants, or 5.5%, were current smokers. Fifty-eight women, or 23.3% of the female sample, had reported taking hormone replacement therapy in 2003. The average CESD depression score among the HSS participants was 6.6 (SD: 0.28), well below the standard cutoff of 16 used to indicate severe levels of symptoms [179].

A significant proportion of the HSS sample reported being exposed to one or more early-life stressors. Figure 3 shows the distribution of reported ELS categories in the sample. Of the 202 people who reported any ELS exposure, 111 (55%) reported one stress category, 55 (27.2%) reported two categories, 25 (12.4%) reported three categories, and 11 people (5.5%) reported four or more. Seventy-six people (14%) had missing information for all possible ELS categories. Although the maximum number of possible categories was seven, no participant reported experiencing all seven – the maximum observed number (in one person) was six. The most commonly reported ELS category was parents arguing (n=116), followed by having a harsh parent (n=101) and a parent dying (n=82). Many of the categories were correlated, with correlation coefficients ranging between .02 and .26 (this highest correlation was between "parents argued" and "had a harsh parent"). However, as has been found previously in the literature, no ELS category came close to perfectly predicting experience of another ELS category.
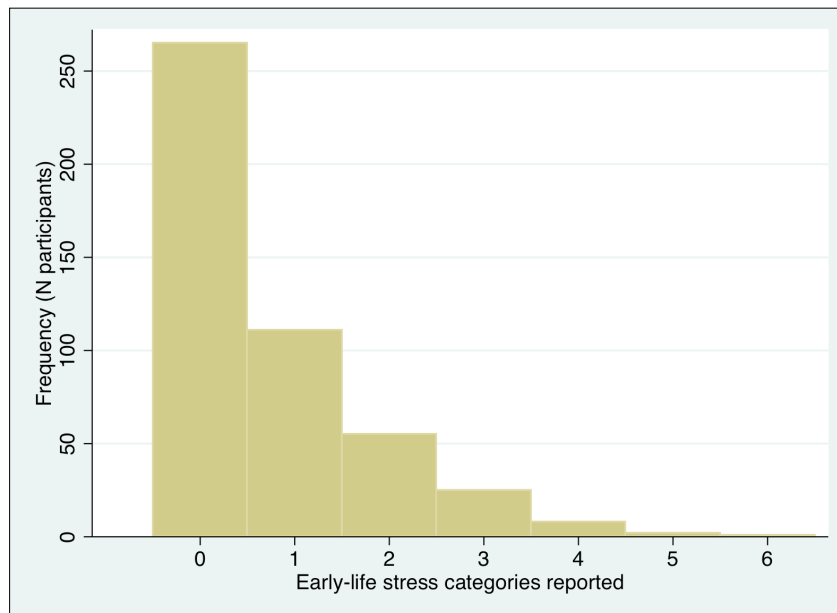

Figure 3. Distribution of early-life stressors in the HSS study sample.

A substantial proportion of the analytic sample also had a history of psychological distress episodes during adulthood, despite the HSS exclusion criteria for recent psychiatric diagnoses or current psychotropic medications. Figure 4 shows the distribution. Overall (i.e., irrespective of early-life stress status), 19.6% (n=83) participants experienced three or more episodes of GHQ scores ≥5, 20.1% (n=85) experienced two such episodes, and 26.2% (n=111) participants experienced one such episode. More than one-third of the participants (34.2%, n=145) experienced no episodes of GHQ scores ≥5 over the six Whitehall waves.
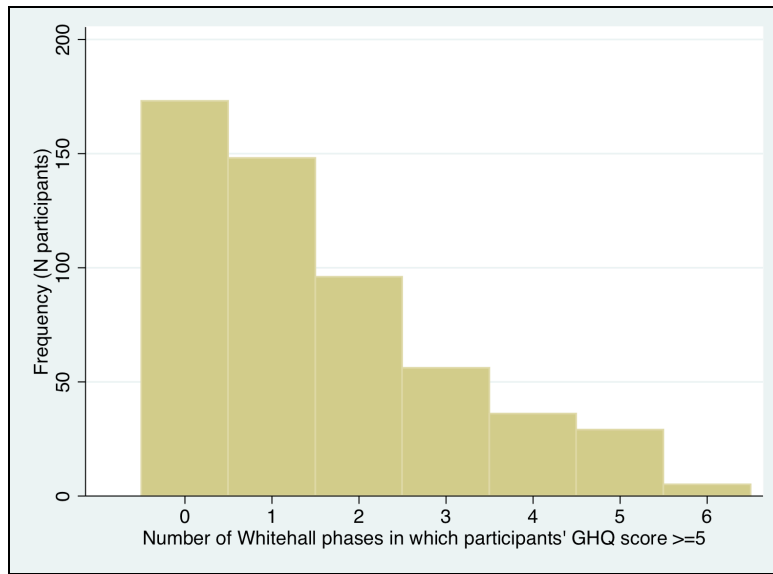
Figure 4. Distribution of GHQ-28 "caseness" episodes in the HSS study sample

Cortisol and IL-6 responses to the stress tasks were, on average, very moderate in size, although highly variable. These moderate responses are probably partly due to the fact that the challenge tasks utilized in the Heart Scan Study are not as psychologically stressful as other stress tasks, and partly because the timing of the laboratory sessions meant that many participants' diurnal cortisol rhythms were in their decreasing phase, such that any temporary rise in cortisol could have been counterbalanced by the concomitant diurnal decrease. Average cortisol levels (unlogged) at baseline were 6.73 nmol/L (SD: 1.59); they subsequently rose an average of 1.07 nmol/L (SD: 1.34) from baseline to immediately post-task, although the maximum cortisol values for nearly 32% of the sample actually occurred *at* baseline. Mean IL-6 values at baseline (unlogged) were 1.18 pg/ML (SD: 1.76). IL-6 levels rose an average of 1.30 pg/mL (SD: 1.48) from baseline to 75 minutes post-task.

Bivariable analyses. Key sociodemographic and biological characteristics of the Heart Scan Study participants, separated by ELS/distress status, are presented in Table 4. ELS-exposed participants were significantly more likely than non-ELS-exposed participants to have experienced any psychological distress episodes (OR=1.81, *p*=.004) and marginally more likely to have experienced three or more episodes (OR=1.45; *p*=.098). Mean GHQ scores for each ELS/distress group at each wave are shown in Figure 3. Across the six waves, the combined non-ELS group had an average GHQ score of 2.55, ELS/no-distress participants had an average score of 0.47, ELS/minimal-distress participants had an average score of 2.55, and ELS/recurrent-distress participants had an average score of 8.74.

Table 4. Sociodemographic and other characteristics of HSS participants, by ELS/distress group

Characteristic	Non-ELS (N = 265)	ELS/ no-distress (N=52)	ELS/ minimal-distress (N = 99)	ELS/ recurrent-distress (N = 51)
Age in years (mean(SD))	62.1 (5.58)	64.6 (5.71)	63.2 (5.36)	61.7 (5.05)
Female, N (%)	111 (41.9%)	29 (55.8%)	42 (42.1%)	32 (62.8%)
Last employment grade, N (%)				
High	122 (46.0%)	14 (26.9%)	33 (33.3%)	21 (41.18%)
Intermediate	104 (39.3%)	22 (42.3%)	45 (45.5%)	19 (37.25%)
Low	39 (14.7%)	16 (30.8%)	21 (21.2%)	11 (21.6%)
Body Mass Index (mean (SD))	25.8 (4.11)	25.0 (3.60)	25.8 (3.43)	26.45 (4.41)
Current smoker, N (%)	12 (4.53%)	0 (0.0%)	9 (9.09%)	4 (7.84%)
Reported HRT in 2003, N (%)	21 (7.9%)	3 (5.8%)	15 (15.2%)	7 (13.7%)
CESD score (mean (SD))	5.81 (5.81)	3.63 (3.33)	6.23 (5.47)	11.73 (8.42)

Participants in the ELS/recurrent-distress category were more likely to be female compared to participants in the non-ELS (p=.054) and ELS/minimal-distress (p<0.01) groups. There were no differences in proportion female between the other groups. The ELS/minimal-distress group was more likely than the non-ELS group to have a lower average employment grade (p=0.001). The ELS/no-distress and ELS/minimal-distress groups tended to be older on average than the non-ELS group (ps<0.10), but no other groups differed by age. Task stressfulness ratings indicated that the majority of participants in all groups found the tasks to be at least somewhat stressful, with no statistically significant group differences (Pearson's χ^2 p=0.93).

Table 5 presents the various ELS categories (parental death, physical abuse, etc.) and their respective frequencies in each ELS/distress group. Columns do not add to 100% because many individuals were exposed to more than one category. There were no significant differences in the proportion of each ELS/distress group exposed to the individual ELS categories except that the ELS/recurrent-distress group was more likely to have experienced a parent's death (Pearson's χ^2 p = 0.027; all other ps>0.24).

Table 5. Frequencies of self-reported early-life stress (ELS) categories, by ELS/distress group

ELS Type	Non-ELS (N = 265)	ELS/ no-distress (N=52)	ELS/ minimal-distress (N = 99)	ELS/ recurrent-distress (N = 51)
Physical abuse	0	2 (3.85%)	9 (9.09%)	3 (5.88%)
1+ years of maternal separation/orphanage	0	12 (23.0%)	16 (16.16%)	11 (2156%)
Parental death	0	10 (19.23%)	28 (28.28%)	22 (43.13%)
Family substance abuse/mental illness	0	8 (15.38%)	12 (12.12%)	9 (17.65%)
Parental divorce	0	3 (5.77%)	4 (4.04%)	2 (3.92%)
Parental conflict	0	26 (50.0%)	55 (55.55%)	24 (47.05%)
Harsh parenting	0	18 (34.62%)	44 (44.44%)	26 (50.98%)

3.1 Aim 1 Analyses: Piecewise growth curve analysis of cortisol responses

3.1.1 Main analyses

Average raw cortisol values for the four groups are shown in Figure 5. Plots for the non-ELS, ELS/no-distress and ELS/minimal-distress groups show a moderate increase in cortisol from baseline to immediately post-task (or 20 min post-task). This was followed by a gradual decline during the recovery period, with average final cortisol levels lower than those at baseline. Compared to the non-ELS group, the ELS/no- and minimal-distress groups' cortisol responses appear higher and more prolonged, most clearly during the recovery period. In contrast, the ELS/recurrent-distress group did not show an increase in cortisol during the reactivity period, instead remaining flat or declining over both reactivity and recovery periods.

Figure 5. Cortisol response slopes, by ELS/distress group
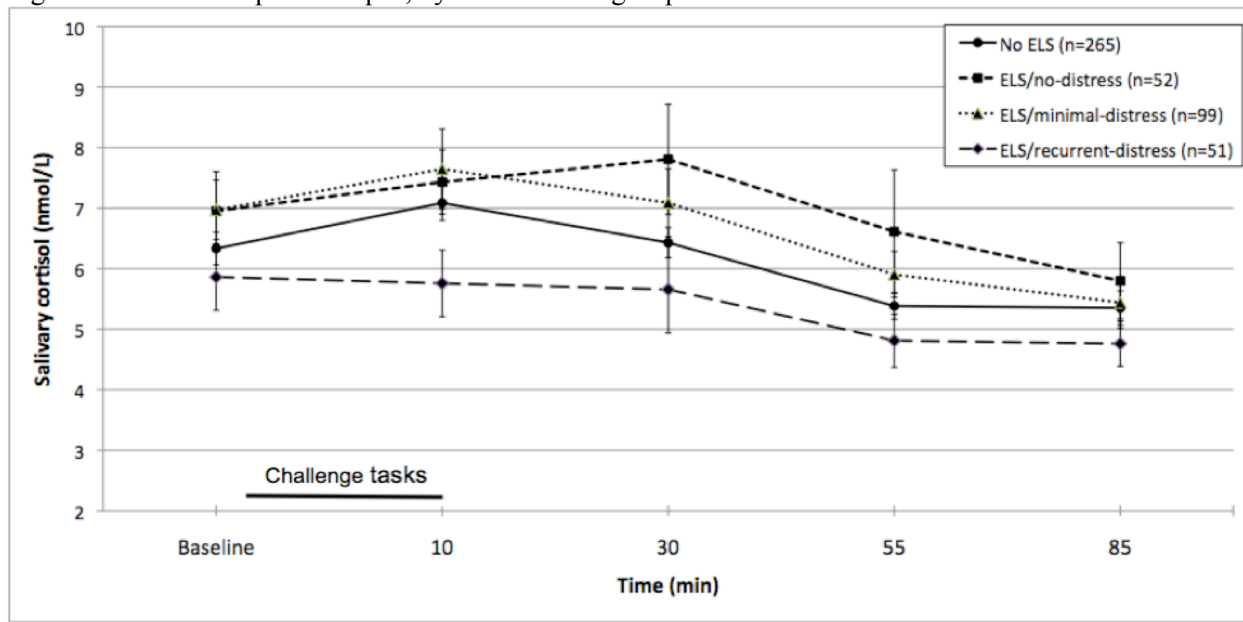
Results from multivariate piecewise growth curve models are shown in Table 6. The model 1 intercept indicates that the mean baseline value for log-cortisol, adjusted for time of visit, was 2.03 nmol/L. Baseline cortisol levels were significantly lower in the afternoon visits compared to the morning visits, but time of visit did not affect cortisol responses to stress. The mean baseline value for the morning sessions, in raw cortisol nmol/L, was 7.86 nmol/L (SD: 5.25); mean baseline value for the afternoon sessions was 5.85 nmol/L (SD: 4.14).) Log-cortisol levels increased significantly during the reactivity period, by an average of .007 nmol/L per minute from baseline (model 1, reactivity). After the tasks ended, log-cortisol declined from its peak by an average of .005 nmol/L per minute (model 1, recovery), but this decline decreased in magnitude by an average of .00003 nmol/L per minute over the recovery period (model 1, recovery^2). Standard deviations for each random parameter in the model are shown under "Random-effects parameters" in Table 4. The intercept, reactivity, recovery, and recovery^2 random parameters indicate the amount of between-individual variability in cortisol baseline value or slopes during the two time periods.

Table 6. Fixed effects and random parameter estimates for piecewise growth curve models of reactivity and recovery levels of log cortisol

	Model 1 Est (SE)	Model 2 Est (SE)	Model 3 Est (SE)
Fixed effects, β (SE)			
Intercept	2.029 (0.029)	2.001 (.025)	1.808 (.151)
Time of visit	−.204 (.034)[a]	−.209 (.026)[a]	−.227 (.026)[a]
Reactivity (linear)	.007 (.001)[a]	.010 (.002)[a]	.010 (.002)[a]
Recovery (linear)	−.005 (.0006)[a]	−.007 (.0006)[a]	−.007 (.0006)[a]
Recovery^2 (quadratic)	.00003 (.000007)[a]	.00004 (.000007)[a]	.00004 (.00001)[a]
ELS/low-distress		.083 (.035)[c]	.054 (.033)
ELS/recurrent-distress		−.070 (.044)	−.064 (.056)
ELS/low-distress by reactivity		−.002 (.002)	−.001 (.002)
ELS/low-distress by recovery		.003 (.001)[c]	.003 (.001)[c]
ELS/low-distress by recovery^2		−.00004 (.00001)[b]	−.00004 (.00001)[b]
ELS/recurrent-distress by reactivity		−.011 (.003)[b]	−.011 (.003)[a]
ELS/recurrent-distress by recovery		.002 (.001)	.002 (.002)
ELS/recurrent-distress by recovery^2		.000004 (.00003)	.000005 (.00003)
Age			.007 (.003)[c]
Gender			−.002 (.032)
Civil service grade			.038 (.009)[a]
BMI			−.012 (.004)[a]
Current smoker			−.180 (.051)[a]
Random-effects parameters			
Standard deviations			
Intercept	.429	.413	.403
Reactivity	.023	.023	.023
Recovery	.011	.011	.011
Recovery^2	.0001	.0001	.0001
Correlations			
Intercept/recovery	-.14	−.45	−.47
Reactivity/recovery	.50	.50	.50
Reactivity/recovery^2	-.61	−.59	−.59
Recovery/recovery^2	-.87	−.88	−.88
-2 x log likelihood	1101.11	894.96	863.50

Models 2 and 3 explored group differences in mean log-cortisol for baseline values and growth trajectories during the reactivity and recovery periods. The ELS/low-distress group had significantly higher baseline log-cortisol levels (mean=1.95 nmol/L; *p*=.052) compared to the non-ELS group (mean=1.88 nmol/L), although this difference was diminished after adjustment

for covariates. Examination of the group × reactivity interaction vectors indicated that the ELS/recurrent-distress group differed significantly from the non-ELS group for cortisol slope during the reactivity period. ELS/recurrent-distress participants had a blunted log-cortisol response to the stressor tasks, differing from the non-ELS group's response by an average of −.001 (.010 − .011) nmol/L per minute. The ELS/low-distress and non-ELS groups' reactivity slopes were parallel, but the ELS/low-distress group's recovery slope remained elevated and did not decline as quickly as the non-ELS group's (model 3, ELS/low-distress by recovery and ELS/low-distress by recovery^2). Post-hoc analyses also showed that the ELS/recurrent-distress and ELS/low-distress groups' reactivity slopes significantly differed from each other.

Gender was not a significant confounder. Older and higher-pay grade participants had significantly higher baseline cortisol values, while smokers and those with higher BMI had lower baseline cortisol. Likelihood ratio tests indicated that model 3 was a significantly better fit than models 1 and 2 (χ^2 $p<.00001$). Results from the nonparametric clustered bootstrap analysis gave inference that was very similar, in comparison to non-bootstrap-based SEs, for all models.

Cortisol AUC_G analyses indicated that the ELS/low-distress group had significantly higher average cortisol output over the laboratory session compared to the ELS/recurrent-distress group ($p<.05$), controlling for the same covariates. Predicted group total cortisol outputs were 496.02 nmol/L (non-ELS), 545.23 nmol/L (ELS/low-distress), and 430.65 nmol/L (ELS/recurrent-distress). The AUC_G statistics comparing the ELS/recurrent-distress and ELS/low-distress groups to the non-ELS group did not reach significance ($p<.20$).

3.1.2 Sensitivity Analyses

Hormone replacement therapy. Although information on hormone replacement therapy (HRT) usage was not collected in the HSS, approximately half of the women (51.8%) had reported taking HRT in Whitehall wave 7 (2003-04). To assess the sensitivity of my results to this factor, I added HRT usage in 2003-04 (yes or no) as a further control variable to the final model. Hormone therapy usage significantly predicted lower cortisol levels (β= -.18, $p<.001$), but as other studies have found, my results were otherwise unaffected [157].

Current depression symptoms. Depression symptomatology at the HSS data collection differed significantly by ELS/distress group (mean CESD score [SD]: no-ELS group, 5.81 [5.81]; ELS/low-distress group, 5.33 [4.98]; ELS/recurrent-distress group, 11.73 [8.42]). To ensure that my results were not driven by these differences, I ran another model that included CESD score as a covariate. The results remained robust; model coefficients did not change appreciably and CESD score was not a significant predictor of cortisol levels ($p>.30$).

Moderation by gender. As a final sensitivity analysis, I examined whether the observed effects were modified by gender [157]. Because testing an overall three-way ELS × distress × gender term was not possible due to the analytic complexity of the model, I instead stratified the models by gender. Visually, these results showed some evidence of effect modification (Figure 6). In both men and women, the ELS/recurrent-distress groups exhibited very blunted cortisol responses, remaining essentially flat or declining across the laboratory session, although baseline values were higher among men. Among women, as in the combined sample, the ELS/low-distress group exhibited high, prolonged cortisol levels across both response and recovery periods in comparison to the other two groups. Among men, the ELS/low-distress

group exhibited a mean cortisol response curve that was higher than the ELS/recurrent-distress group's, but very similar to the non-ELS group's.

Statistically, the gender-stratified models also differed. Among men, of the model terms for log-cortisol baseline and response periods, only the term for a blunted reactivity slope ($p<.001$) in the ELS/recurrent-distress group was significant. Among women, model terms for heightened baseline cortisol in the ELS/low-distress group ($p<.05$), and heightened reactivity ($p<.05$) and flatter recovery ($p<.05$) slopes for the ELS/low-distress group, were significant. There was a trend indicating that the female ELS/recurrent-distress group's cortisol slopes were blunted compared to the no-ELS group's, but it did not reach significance ($p=.12$).
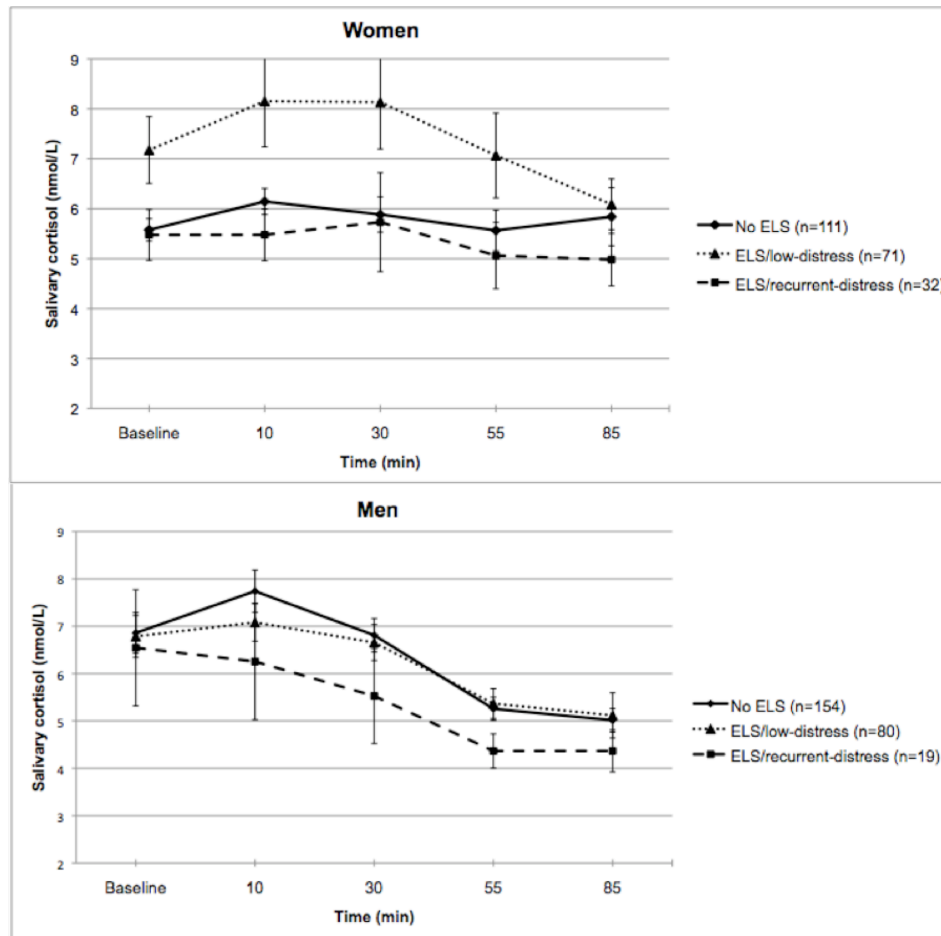
Figure 6. Cortisol response curves by ELS/distress group, stratified by gender.

3.2 Aims 2 and 3 analyses

3.2.1 Aim 2: Group-based trajectory modeling of cortisol and IL-6

Model fitting: Cortisol data. Table 7 shows the results from the initial group-based trajectory modeling for the cortisol data. I iteratively specified a maximum of six trajectory groups, using a combination of linear and quadratic forms for each trajectory. All models were tested initially using solely linear forms for each trajectory. Subsequently, all trajectories were

specified as quadratic and the results examined for non-significant quadratic terms, which were then eliminated, typically resulting in some trajectories being specified as quadratic and some as linear. Preliminary model selection was accomplished through examination of the BIC values and use of the 2(delta BIC) criterion, which compared the fit of a larger (more trajectories) model to that of a smaller (fewer trajectories) model. Some heuristic judgment about model selection was also required, as BIC values continued to increase with each additional trajectory group specified, up to the tested maximum of six.

Table 7. Model solutions and BICs for cortisol data					
Number of groups	Form of model[*]	Log likelihood	BIC	Null Model	2(deltaBIC)[**]
1	1	-1776.60	-1786.00	-	-
2	12	-1296.22	-1318.14	1	935.72
3	112	-1094.36	-1126.32	2	383.64
4	1122	-968.53	-1013.46	3	225.72
5	11112	-915.83	-970.98	4	84.96
6	111121	-907.97	-944.84	5	52.28

[*]1 indicates a linear model; 2 indicates a quadratic model. E.g., "12" means that the first trajectory group's model had a linear form and the second group's model had a quadratic form.
[**]Delta(BIC) = 2 x (BIC of more complex model – BIC of null model)

Since this analysis was purely exploratory, I used a combination of subject-matter expertise and established criteria to finalize the model selection. I decided that the best model was a four-trajectory group solution for the following reasons: First, the improvement in BIC values reached a plateau and flattened out at approximately four groups (shown in Figure 7). Second, at a five-group solution, one of the trajectory groups contained only 1.15% of the sample's participants, which seemed unstable – Nagin [174] recommends that final models contain groups with a minimum of 5% of the sample. The magnitude of the 2(deltaBIC) value for the five-group solution (84.96) was also considerably smaller than that for the four-group solution (225.72). Third, comparing the four-group solution to a three-group solution resulted in a 2(delta BIC) value of 225.72, which corresponds to very strong evidence that the larger (four-group) model was a better fit.
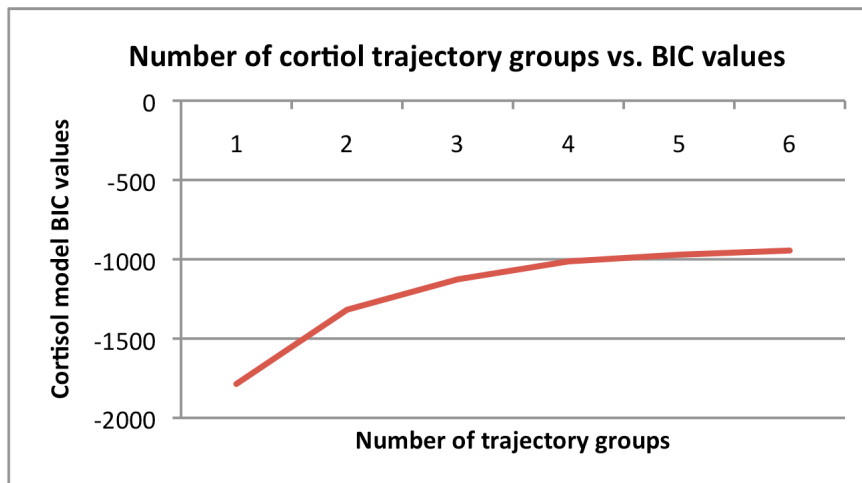

Figure 7. Cortisol trajectory model BIC values plotted against the number of groups specified in the model
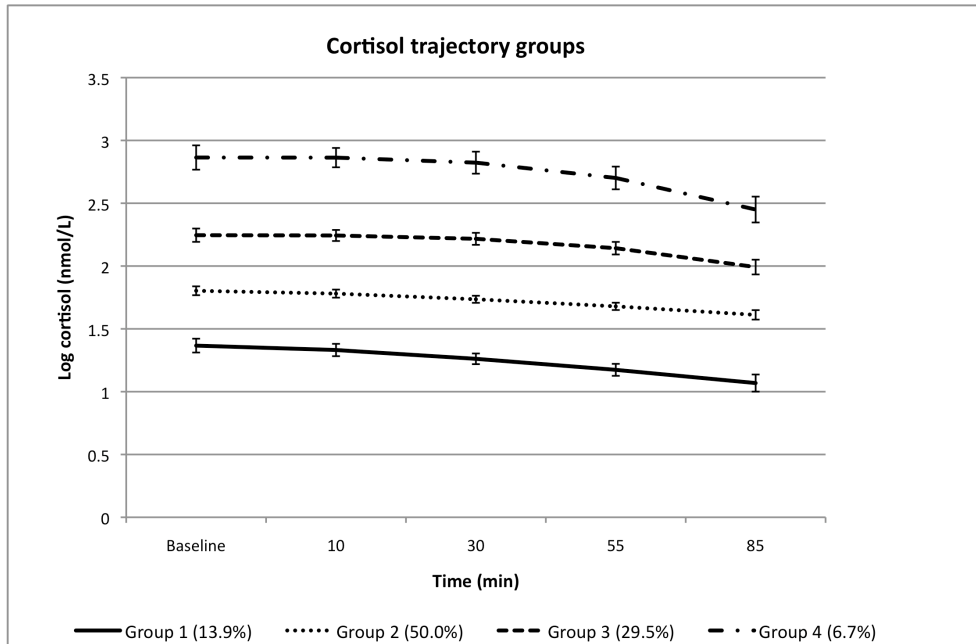
Figure 8. Predicted cortisol group trajectories and 95% confidence intervals.

The final four-group model consisted of the following cortisol trajectory groups:

1. A linear trajectory (Group 1 or "low-declining") that started out with baseline log-cortisol levels of 1.37 nmol/L and decreased steadily from there.
2. A second linear trajectory (Group 2 or "middle-flat") that had baseline values of 1.80 nmol/L and very slightly decreased throughout the laboratory session.
3. A quadratic trajectory (Group 3 or "higher-flat") that had baseline values of 2.25 nmol/L, a very small increase during the reactivity period, and then declined during the recovery period.
4. A second quadratic trajectory (Group 4 or "high-declining") that had the highest baseline values (2.86 nmol/L) of any group and a slight increase during the reactivity period, and then declined substantially during the recovery period.

Groups 2 and 3 contained the largest proportions of the sample, with 50.0% and 29.5%, respectively, while Group 1 contained 13.9% and Group 4 contained 6.7% of the sample. Figure 8 displays the predicted group trajectories and 95% confidence intervals around each trajectory, using the Taylor series expansion estimates. The overall increase in participants' cortisol levels in response to the acute stressor is not very apparent in this graph, as the values are on the log scale.

Table 8 contains the model parameters and group percentages, plus predicted values and 95% confidence intervals (both Taylor series and bootstrapped) around those values at each measurement, for each cortisol trajectory group. The Taylor series and bootstrapped 95% confidence intervals were essentially identical, with the bootstrapped estimates often slightly narrower than the Taylor series estimates. Box plots of the bootstrapped cortisol model betas and group percentages (π_j) are provided in the Appendix.

Table 8. Group-based trajectory model statistics for cortisol data*					
Model parameters and group percentages					
Group	**Group label**	**Intercept**	**Linear term**	**Quadratic term**	**Group percentage**
1	Low-declining	1.366	-0.035		13.9%
2	Middle-flat	1.803	-0.023		50.0%
3	Higher-flat	2.245	0.001	-0.004	29.5%
4	High-declining	2.864	0.005	-0.006	6.7%
Predicted cortisol values and 95% confidence intervals at each measurement					
		Group 1	**Group 2**	**Group 3**	**Group 4**
Baseline	Mean	1.366	1.803	2.246	2.864
	Taylor 95% CI	(1.31, 1.42)	(1.77, 1.84)	(2.19, 2.30)	(2.77, 2.96)
	Bootstrap 95% CI	(1.32, 1.42)	(1.77, 1.83)	(2.20, 2.28)	(2.78, 2.95)
10 min	Mean	1.331	1.781	2.243	2.863
	Taylor 95% CI	(1.28, 1.38)	(1.75, 1.81)	(2.20, 2.29)	(2.79, 2.94)
	Bootstrap 95% CI	(1.29, 1.38)	(1.76, 1.81)	(2.21, 2.27)	(2.80, 2.92)
30 min	Mean	1.262	1.735	2.217	2.823
	Taylor 95% CI	(1.22, 1.30)	(1.71, 1.76)	(2.17, 2.26)	(2.74, 2.91)
	Bootstrap 95% CI	(1.23, 1.30)	(1.72, 1.76)	(2.18, 2.25)	(2.76, 2.90)
55 min	Mean	1.174	1.679	2.142	2.701
	Taylor 95% CI	(1.13, 1.22)	(1.65, 1.71)	(2.09, 2.19)	(2.61, 2.79)
	Bootstrap 95% CI	(1.14, 1.22)	(1.66, 1.70)	(2.11, 2.18)	(2.63, 2.78)
85 min	Mean	1.069	1.611	1.992	2.450
	Taylor 95% CI	(1.00, 1.14)	(1.57, 1.65)	(1.93, 2.05)	(2.35, 2.55)
	Bootstrap 95% CI	(1.01, 1.13)	(1.58, 1.64)	(1.94, 2.04)	(2.36, 2.55)

* Again, inference stemming from these confidence intervals should be treated with great caution, as parameter estimates are not smooth functionals of the data (i.e., no central limit theorem applies here).

Parametric bootstrap sensitivity analysis. As described in Chapter 2, I also performed a preliminary sensitivity analysis for the parametric bootstrap procedure, to evaluate the stability of those results. The model fitting results are shown in Table 9. In each of the 10 replicate datasets, a four-group mixture solution was the best fit. However, the order and degree of the polynomials for each group varied substantially from replicate to replicate, underscoring the inherent instability and non-robustness of the Nagin method – even in a "best case" scenario, where one assumes that the originally specified model is correct.

Table 9. Sensitivity analysis bootstrap: Model solutions and BICs for cortisol data. Best-fitting trajectory model solution for each replicate dataset is highlighted in bold.				
Replicate #1				
Number of groups	Form of model	BIC	Null Model	2(deltaBIC)
1	1	-1887.69	-	-
2	11	-1399.88	1	975.62
3	111	-1225.90	2	347.96
4	**1112**	**-1071.07**	**3**	**309.66**
5	11112	-1080.52	4	-18.9
6	111111	-1089.07	5	-17.1

Replicate #2				
Number of groups	Form of model	BIC	Null Model	2(deltaBIC)[**]
1	1	-1795.34	-	-
2	11	-1356.32	1	878.04
3	112	-1198.09	2	316.46
4	**1112**	**-1074.02**	**3**	**248.14**
5	11112	-1081.31	4	-14.58
6	111111	-1089.27	5	-15.92
Replicate #3				
Number of groups	Form of model	BIC	Null Model	2(deltaBIC)[**]
1	1	-1755.49	-	-
2	12	-1354.90	1	801.18
3	112	-1203.53	2	302.74
4	**1122**	**-1072.85**	**3**	**261.36**
5	11122	-1082.19	4	-18.68
6	111122	-1091.64	5	-18.9
Replicate #4				
Number of groups	Form of model	BIC	Null Model	2(deltaBIC)[**]
1	1	-1902.96	-	-
2	12	-1397.78	1	1010.36
3	112	-1263.81	2	267.94
4	**2122**	**-1131.77**	**3**	**264.08**
5	11122	-1139.96	4	-16.38
6	221122	-1152.98	5	-26.04
Replicate #5				
Number of groups	Form of model	BIC	Null Model	2(deltaBIC)[**]
1	1	-1822.67	-	-
2	12	-1361.20	1	922.94
3	112	-1190.20	2	342
4	**1122**	**-1039.75**	**3**	**300.9**
5	11122	-1049.05	4	-18.6
6	111212	-1054.27	5	-10.44
Replicate #6				
Number of groups	Form of model	BIC	Null Model	2(deltaBIC)[**]
1	1	-1849.77	-	-
2	12	-1367.71	1	964.12
3	112	-1227.13	2	281.16
4	**1112**	**-1086.05**	**3**	**282.16**
5	11122	-1096.93	4	-21.76
6	111122	-1106.37	5	-18.88
Replicate #7				
Number of groups	Form of model	BIC	Null Model	2(deltaBIC)[**]
1	1	-1814.45	-	-
2	12	-1366.08	1	896.74
3	112	-1200.91	2	330.34
4	**1121**	**-1047.15**	**3**	**307.52**
5	11121	-1051.31	4	-8.32
6	111121	-1058.26	5	-13.9

Replicate #8				
Number of groups	Form of model	BIC	Null Model	2(deltaBIC)**
1	1	-1913.58	-	-
2	12	-1386.00	1	1055.16
3	112	-1264.66	2	242.68
4	**1111**	**-1100.60**	**3**	**328.12**
5	11111	-1108.32	4	-15.44
6	111111	-1117.76	5	-18.88

Replicate #9				
Number of groups	Form of model	BIC	Null Model	2(deltaBIC)**
1	1	-1864.01	-	-
2	12	-1373.20	1	981.62
3	112	-1218.88	2	308.64
4	**1122**	**-1057.46**	**3**	**322.84**
5	11122	-1062.89	4	-10.86
6	111122	-1070.77	5	-15.76

Replicate #10				
Number of groups	Form of model	BIC	Null Model	2(deltaBIC)**
1	1	-1798.34	-	-
2	12	-1380.09	1	836.5
3	112	-1209.83	2	340.52
4	**1122**	**-1074.14**	**3**	**271.38**
5	11122	-1082.27	4	-16.26
6	111112	-1093.99	5	-23.44

Model fitting: IL-6 data. Table 10 shows the results from the initial group-based trajectory modeling for the IL-6 data. I iteratively specified a maximum of seven trajectory groups, using a combination of linear and quadratic forms for each trajectory. All models were again tested initially using linear forms for each trajectory. Subsequently, all trajectories were specified as quadratic and the results examined for non-significant quadratic terms, which were eliminated. None of the quadratic terms were significant, indicating that linear model forms were a better fit for the IL-6 data. Again, preliminary model selection was accomplished through examination of the BIC values and the 2(delta BIC) criterion.

Table 10. Model solutions and BICs for IL-6 data					
Number of groups	Form of model	Log likelihood	BIC	Null Model	2(deltaBIC)
1	1	-1768.69	-1778.11	-	-
2	11	-1221.32	-1240.15	1	1075.92
3	111	-891.72	-919.96	2	640.38
4	1111	-759.47	-797.13	3	245.66
5	**11111**	**-700.31**	**-747.38**	**4**	**118.32**
6	111111	-647.71	-704.20	5	86.36
7	1111111	-601.38	-667.28	6	73.84

Similarly to the cortisol data, BIC values for the IL-6 data continued to increase with increasing complexity of the model. However, once again, the improvement in BIC values reached a plateau at approximately five groups (shown in Figure 9). The group sizes also became increasingly unstable with the largest (six- and seven-group) solutions. At a six-group

solution, there were two groups containing approximately 5% of the sample – 4.68% and 5.68%, or roughly 25 and 30 people, respectively. Comparing the five-group solution to a four-group solution resulted in a 2(delta BIC) value of 118.32, which again corresponds to very strong evidence that the larger (four-group) model was a better fit. Therefore, for the same reasons as listed above for the cortisol data, I decided that the five-group solution was the best choice.
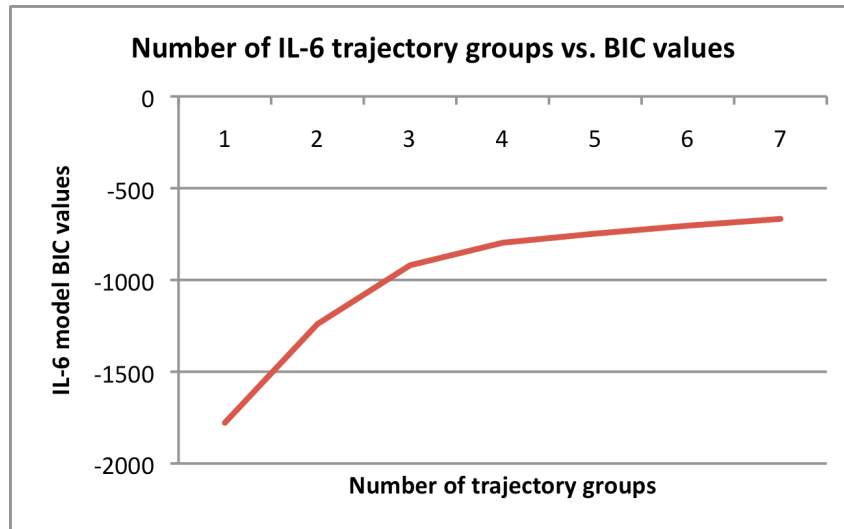

Figure 9. IL-6 trajectory model BIC values plotted against the number of groups specified in the model.

The final five-group solution consisted of groups that were strikingly similar to each other in shape. All trajectory groups remained essentially flat during the reactivity period (baseline to 10 min), then increased slightly but steadily for the remainder of the laboratory session time period (see Figure 10). The only major difference between the groups was their baseline values. Mean predicted baseline log IL-6 values in the five groups ranged from -0.597 pg/ml in the lowest group (group 1) to 1.286 pg/ml in the highest group (group 5).

Groups 2 and 3 contained the largest proportions of the sample, with 32.4% and 30.4%, respectively, while Group 1 contained 12.6%, Group 4 contained 15.0%, and Group 5 contained 9.5% of the sample. Figure 10 displays the predicted group trajectories and 95% confidence intervals around each trajectory (using the Taylor series expansion estimates).
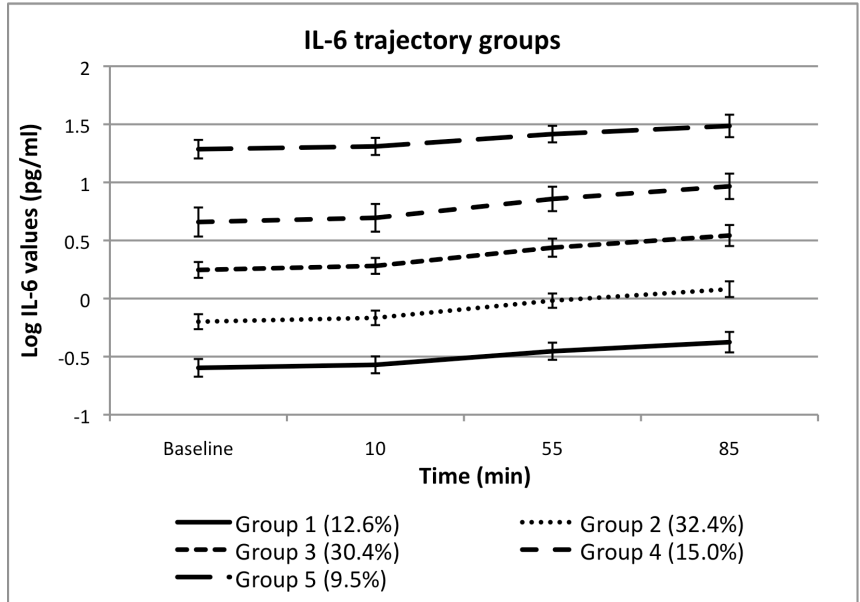
Figure 10. Predicted IL-6 group trajectories and 95% confidence intervals.

Table 11. Group-based trajectory model statistics for IL-6 data			
Model parameters and group percentages			
Group	Intercept	Linear term	Group percentage
1	-0.597	0.026	12.6%
2	-0.199	0.033	32.4%
3	0.246	0.035	30.4%
4	0.659	0.036	15.0%
5	1.286	0.024	9.5%
Predicted values and 95% confidence intervals at each measurement			

		Baseline	10 min	55 min	85 min
Group 1	Mean	-0.597	-0.571	-0.453	-0.376
	Taylor 95% CI	(-0.67, -0.52)	(-0.64, -0.50)	(-0.53, -0.38)	(-0.46, -0.29)
	Bootstrap 95% CI	(-0.65, -0.54)	(-0.61, -0.53)	(-0.56, -0.48)	(-0.51, -0.39)
Group 2	Mean	-0.199	-0.166	-0.018	0.081
	Taylor 95% CI	(-0.26, -0.13)	(-0.23, -0.10)	(-0.08, 0.04)	(0.01, 0.15)
	Bootstrap 95% CI	(-0.23, -0.16)	(-0.19, -0.14)	(-0.13, -0.07)	(-0.06, 0.02)
Group 3	Mean	0.246	0.281	0.438	0.542
	Taylor 95% CI	(0.18, 0.31)	(0.21, 0.35)	(0.36, 0.52)	(0.45, 0.63)
	Bootstrap 95% CI	(0.22, 0.28)	(0.25, 0.31)	(0.33, 0.38)	(0.40, 0.48)
Group 4	Mean	0.658	0.695	0.858	0.966
	Taylor 95% CI	(0.53, 0.78)	(0.58, 0.81)	(0.75, 0.96)	(0.86, 1.08)
	Bootstrap 95% CI	(0.61, 0.71)	(0.66, 0.74)	(0.73, 0.80)	(0.81, 0.91)
Group 5	Mean	1.286	1.309	1.416	1.486
	Taylor 95% CI	(1.21, 1.37)	(1.24, 1.38)	(1.34, 1.49)	(1.39, 1.58)
	Bootstrap 95% CI	(1.23, 1.34)	(1.27, 1.35)	(1.32, 1.39)	(1.35, 1.48)

* Again, inference stemming from these confidence intervals should be treated with great caution, as parameter estimates are not smooth functionals of the data (i.e., no central limit theorem applies here).

Table 11 contains the model parameters and group percentages, plus predicted values and 95% confidence intervals (both Taylor series and bootstrapped) around those values at each measurement, for each IL-6 trajectory group. Again, the confidence intervals calculated using the two methods were quite similar, with an even more noticeable trend for the bootstrapped intervals to be narrower than the Taylor series intervals. Box plots of the bootstrapped IL-6 model betas and group percentages (π_j) are provided in the Appendix.

Posterior probabilities of group membership: Cortisol and IL-6 data. Once the final models for the cortisol and IL-6 data were established, I generated the participants' posterior probabilities of membership in each cortisol and IL-6 group. The *average* posterior probabilities for each trajectory group (both cortisol and IL-6) were close to 1.0, ranging from 0.89 to 0.95, indicating a very good model fit. These posterior probabilities were used in conjunction with the group membership probabilities (π) to calculate the odds of correct classification, which Nagin [174] uses – in combination with P_j (the proportion of the sample actually classified in group j) and the average posterior probabilities themselves – as diagnostics of assignment accuracy. Table 12 lists the trajectory groups for both cortisol and IL-6, as well as these diagnostics for each group. The P_j and odds of correct classification were also high for all groups, particularly the highest groups (4 and 5) in the cortisol and IL-6 data, respectively. Together, these diagnostic assessments indicated that trajectory group assignments from the group-based trajectory models were fairly stable and accurate for both the cortisol and IL-6 data.

Table 12. Diagnostics of assignment accuracy for cortisol and IL-6 data				
Cortisol data				
Group	π	Proportion classified in group j	Average Posterior Probability	Odds of correct classification*
1	.139	.133	.928	79.8
2	.500	.525	.924	12.2
3	.295	.278	.918	26.8
4	.067	.065	.941	222.1
IL-6 data				
Group	π	Proportion classified in group j	Average Posterior Probability	Odds of correct classification*
1	.126	.118	.910	70.1
2	.324	.346	.895	17.8
3	.304	.300	.894	19.3
4	.150	.142	.893	47.3
5	.095	.094	.954	197.6

*Odds of correct group j classification: $((\text{Average Posterior Prob.}_j / 1 - \text{AvePP}_j) / (\pi_j / 1 - \pi_j)$

Dual trajectory modeling. For the second part of Aim 2, my goal was to determine whether membership in a given IL-6 trajectory group was associated with membership in a particular cortisol trajectory group. I used unconditional dual-trajectory analysis for this aim, incorporating the model specifications (shape and number of trajectory groups) used in the single-trajectory approach.

Dual-trajectory model estimation resulted in a BIC of -1791.95, AIC of -1703.97, and a log likelihood of -1662.97. Table 13 displays the conditional probabilities of group

membership for both IL-6 and cortisol data (panels a and b), as well as the joint probabilities of membership (panel c). In other words, panel (c) enumerates the probabilities all possible combinations of cortisol and IL-6 trajectory groups. In panels (a) and (b), each column sums to 100%; in panel (c), the entire panel's percentages sum to 100%.

The principal finding, apparent when examining Table 13, is that membership in a given cortisol or IL-6 group is not perfectly – or even close to perfectly – predictive of group membership in the other biomarker. Despite the biological interrelationship between cortisol and IL-6, there is considerable heterogeneity in how participants' acute stress responses of these biomarkers covary. The highest conditional probability in either biomarker is 52%, and most are below 40%.

Nevertheless, certain patterns do emerge in the conditional and joint probabilities. Examining the probability of IL-6 group membership given cortisol group membership (panel a), it becomes apparent that participants in the lowest cortisol group (Group 1 or "low-decliners") are very unlikely to be in the lowest IL-6 group. Although probabilities of being in any of the extreme groups are comparatively low in general, cortisol low-decliners are 4.2 times as likely to be in the highest IL-6 group (Group 5) as they are to be in IL-6 Group 1. Furthermore, the cortisol low-decliners' probability of being in one of the *two* highest IL-6 groups is 31%. On the other hand, people in the highest cortisol group ("high-decliners") are more likely to be in the lower IL-6 groups: Given membership in the highest cortisol group, probability of being in one of the lowest two IL-6 groups is nearly 50%. For people in the middle cortisol groups – the "middle-flats" and "high-flats" – IL-6 group membership probabilities tend to be more evenly distributed.

Table 13. Conditional and joint probabilities of IL-6 and cortisol group membership					

13a. Probability of IL-6 group membership *conditional on* membership in given cortisol group

Probability of membership in IL-6 group:	Cortisol group				
	1	2	3	4	
1	2.38%	9.86%	16.33%	10.53%	
2	27.95%	32.53%	32.88%	39.40%	
3	38.42%	31.32%	27.25%	11.79%	
4	21.13%	15.90%	14.77%	25.82%	
5	10.11%	10.39%	8.77%	12.46%	

13b. Probability of cortisol group membership *conditional on* membership in given IL-6 group

Probability of membership in cortisol group:	IL-6 group				
	1	2	3	4	5
1	3.10%	12.20%	18.20%	17.60%	14.30%
2	45.70%	49.90%	52.30%	46.60%	51.70%
3	44.50%	29.70%	26.80%	25.50%	25.70%
4	6.60%	8.20%	2.70%	10.30%	8.40%

13c. Joint probabilities of cortisol and IL-6 group membership

Cortisol group	IL-6 group				
	1	2	3	4	5
1	0.30%	4.00%	5.40%	3.00%	1.40%
2	4.90%	16.20%	15.60%	7.90%	5.20%
3	4.80%	9.60%	8.00%	4.30%	2.60%
4	0.70%	2.70%	0.80%	1.70%	0.80%

In panel (b), it again becomes clear that most participants are clustered in the middle trajectory groups, for both biomarkers. For example, for each IL-6 group, between 72.1% and 90.2% of those participants are clustered in the middle two cortisol groups. However, there are noteworthy trends. "Opposite" biomarker groups again appear to cluster together. For example, when examining membership in the two most extreme IL-6 groups (1 and 5), people in the *highest* IL-6 group (5) are far more likely – 4.6 times as likely, specifically – than are people in the *lowest* IL-6 group (1) to be in the *lowest* cortisol group. Similarly, given membership in the *lowest* IL-6 group (1), participants are more than twice as likely to be in the *highest* cortisol group compared to the *lowest* cortisol group. In addition, this lowest IL-6 group's combined probabilities of being in the two highest cortisol groups (51.1% total) are much higher than any other IL-6 group's (37.9%, for the second-lowest IL-6 group, being the next highest). Lastly, those people in the highest and lowest IL-6 groups, respectively, are very unlikely to be in the highest and lowest cortisol groups (respective probability of memberships: 8.4% and 3.1%). They are, comparatively, much more likely to be in the *lowest* and *highest* cortisol groups (14.3% and 6.6%, respectively).

The unusualness of following a joint probability of high levels in one biomarker as well as high levels in the other, or low levels in both, is highlighted in panel (c). Only 0.8% of the sample fell into the highest trajectories of both biomarkers, and only 0.3% fell into the lowest trajectories. Nearly 62% of the sample fell into the middle trajectories on both biomarkers. Although still small, the joint probabilities of falling into "opposite" (high vs. low) biomarker trajectories were substantially higher than those of falling into congruent trajectories.

This "opposites attract" trend is biologically plausible, even expected. As described in the Introduction, cortisol is a powerful suppressor of IL-6 production. Therefore, people with higher baseline and acute cortisol responses should be expected to demonstrate lower IL-6 baseline levels and responses. However, again, the most notable finding from the dual-trajectory analysis is the heterogeneity of joint group membership across all levels of cortisol and IL-6.

Predictors of trajectory group membership. For Aim 3, my goal was to examine whether exposure to early-life stress, history of psychological distress, and other key covariates predicted membership in specific cortisol and IL-6 trajectory groups. I was originally going to include smoking status in these covariate models, but there were so few smokers in the sample that the parameter estimates were unstable, leading me to exclude this variable from the final models. Thus, my final models included ELS/distress status as well as age, gender, BMI, civil service employment grade, and time of laboratory visit.

Running the covariate models necessitated selecting a comparison trajectory group for each biomarker. This was a relatively arbitrary choice, although for questions like mine where the primary interest is in identifying atypical patterns, Van Ryzin et al. [171] recommend selecting the "most typical" pattern as the comparison group. I therefore decided to make the comparison group for each biomarker the trajectory group whose mean baseline values were closest to the overall sample's mean baseline for that biomarker. For cortisol, this meant that Group 2 (the "middle-flat" group) was the comparison; this group contained approximately 50% of the sample. For IL-6, the comparison group was Group 3, which contained approximately 30% of the sample.

Table 14. Multivariate logistic model predicting cortisol trajectory membership*

Group	Parameter	Estimate	S.E.	p
1	Constant	-1.082	2.372	0.6484
	ELS/low-distress	0.559	1.535	0.1743
	ELS/recurrent-distress	**2.242**	**1.562**	**0.0703**
	Age	0.996	1.033	0.9031
	BMI	0.999	1.041	0.986
	Gender	0.943	1.400	0.8628
	Civil service grade	**0.806**	**1.132**	**0.0822**
	Time of laboratory visit	**2.577**	**1.495**	**0.0188**
2	(Comparison)	.	.	.
3	Constant	-1.36225	1.79127	0.4470
	ELS/low-distress	1.012	1.318	0.9661
	ELS/recurrent-distress	0.697	1.567	0.4231
	Age	1.041	1.025	0.1009
	BMI	0.949	1.035	0.1259
	Gender	0.944	1.299	0.8270
	Civil service grade	1.102	1.090	0.2595
	Time of laboratory visit	**0.492**	**1.314**	**0.0095**
4	Constant	-4.79906	3.01524	0.1116
	ELS/low-distress	1.040	1.588	0.9327
	ELS/recurrent-distress	0.357	3.213	0.3779
	Age	**1.129**	**1.039**	**0.0015**
	BMI	**0.861**	**1.071**	**0.0290**
	Gender	0.582	1.593	0.2447
	Civil service grade	1.165	1.157	0.2947
	Time of laboratory visit	**0.238**	**1.608**	**0.0025**

*Note that as with the bootstrap results above, inference from this analysis must be regarded with extreme caution.

Table 14 provides the parameter estimates (odds ratios) and standard errors for each covariate predicting membership in the cortisol trajectory groups. Statistically significant and marginally significant parameter estimates are highlighted in bold font. When compared to membership in the "middle-flat" Group 2, membership in cortisol Group 1 ("low-declining") was significantly predicted by time of laboratory visit (OR=2.58, p=0.012), and marginally significantly predicted by ELS/recurrent-distress status (OR=2.42, p=0.07) and civil service grade (i.e., socioeconomic status; OR=0.49, p=0.08), controlling for other covariates. Specifically, participants whose laboratory sessions took place in the afternoon rather than in the morning, as well as participants who had been exposed to both early-life stress and subsequent recurrent problems with psychological distress, were *more* likely to be classified in the low-declining cortisol group than in the middle-flat group. Since civil service grade is reverse-categorized, the parameter indicates that participants of lower socioeconomic status were *less* likely to fall into the low-declining cortisol group.

Only time of laboratory visit predicted membership in the "higher-flat" cortisol group (Group 3). In this case, participants whose visits took place in the afternoon were *less* likely to be in the higher-flat group compared to in the middle-flat group (OR=0.49, p<0.01). The same was true for membership in the "high-declining" cortisol group (Group 4), with an even greater

magnitude (OR=0.24, *p*=0.002). As cortisol levels typically decline across the day, this pattern is biologically logical. Group 4 membership was also predicted by age and BMI: older participants were more likely to fall into the high-declining group than into the middle-flat group (OR=1.13, *p*=0.001), as were participants with a lower BMI (OR=0.86, *p*=0.03).

Group	Parameter	Estimate	S.E.	*p*
	Table 15. Multivariate logistic model predicting IL-6 trajectory membership			
1	Constant	16.2205	2.92826	0.0000
	ELS/low-distress	1.711	1.478	0.1694
	ELS/recurrent-distress	1.023	1.910	0.9714
	Age	**0.862**	**1.039**	**0.0001**
	BMI	**0.747**	**1.057**	**0.0000**
	Civil service grade	**0.753**	**1.135**	**0.0251**
	Gender	1.250	1.451	0.5487
	Time of laboratory visit	0.608	1.446	0.1765
2	Constant	9.48961	2.08385	0.0000
	ELS/low-distress	1.204	1.366	0.5523
	ELS/recurrent-distress	1.307	1.588	0.5629
	Age	**0.931**	**1.027**	**0.0064**
	BMI	**0.840**	**1.039**	**0.0000**
	Civil service grade	**0.780**	**1.103**	**0.0113**
	Gender	1.024	1.332	0.9337
	Time of laboratory visit	1.112	1.328	0.7086
3	(Comparison group)	.	.	.
4	Constant	-3.45093	2.70398	0.2020
	ELS/low-distress	0.601	1.546	0.2423
	ELS/recurrent-distress	1.682	1.727	0.341
	Age	1.037	1.034	0.2815
	BMI	0.967	1.051	0.5005
	Civil service grade	1.029	1.133	0.8201
	Gender	1.417	1.469	0.3649
	Time of laboratory visit	**2.384**	**1.535**	**0.0427**
5	Constant	-3.16324	3.00657	0.2929
	ELS/low-distress	1.112	1.602	0.8222
	ELS/recurrent-distress	1.865	1.862	0.3165
	Age	1.013	1.039	0.7402
	BMI	1.026	1.052	0.6219
	Civil service grade	0.879	1.152	0.365
	Gender	1.275	1.516	0.5597
	Time of laboratory visit	1.110	1.532	0.8062

*Note that as with the bootstrap results above, inference from this analysis must be regarded with extreme caution.

Table 15 provides the parameter estimates and standard errors for each covariate predicting membership in the IL-6 trajectory groups. Again, statistically significant parameters are highlighted in bold font. Age, BMI, and socioeconomic status all predicted membership in the lowest two IL-6 trajectory groups, compared to the middle IL-6 group. Specifically, participants who were older, had higher BMI, and were of lower socioeconomic status were *less* likely to be in the lowest two groups, controlling for other variables.

In the second highest IL-6 trajectory group (Group 4), only time of laboratory visit proved to be a significant predictor, such that participants whose laboratory visits took place in the afternoon were more likely to be in Group 4 when compared to Group 3 (OR=2.34, p=0.04). None of the specified covariates significantly predicted membership in IL-6 group 5. Notably, ELS/distress status was not a significant predictor of any IL-6 group membership.

Once again, the flaws in the group-based modeling approach mean that this analysis should be considered extremely exploratory, and the results interpreted only with great caution.

Chapter 4: Discussion and Next Steps

In this dissertation, I examined the association between experiencing early-life stress, subsequent symptoms of depression and anxiety, and two biological systems that are hypothesized to underlie the effects of early-life stress on such psychological symptoms. This research question builds from a complex theoretical and empirical literature, described in Chapter 1, that seeks to understand how social experiences – including early-life adversity – "get under the skin" and alter aspects of our fundamental biology, alterations which in turn may contribute to the physical and mental health burden in a population. Specifically, I sought to investigate whether early-life stress predicted changes in individuals' cortisol and immune system reactions to an experimental stressor and, if so, whether any such differences in stress reactivity were additionally moderated by the individuals' history of recurrent anxiety and depression symptoms. I examined these questions using a unique dataset that combined longitudinal, prospectively-collected cohort data on the social and psychological experiences of a sample of British white-collar workers with biological data that had been collected on them during an experimental laboratory-based study.

4.1 Summary of the Findings

Aim 1. My findings for Aim 1 – in which I used random-effects growth-curve modeling and area-under-the-curve analyses to examine the association between ELS/distress status and cortisol reactivity – suggest that adults exposed to early-life adversity have dysregulated cortisol reactivity trajectories, but that the pattern of those trajectories differs based on their history of psychological distress during adulthood. ELS-exposed older adults with recurrent psychological distress had blunted cortisol responses compared to non-ELS-exposed adults, as well as decreased overall cortisol output compared to ELS-exposed adults with a history of no or minimal psychological distress. In contrast, these ELS-exposed adults with minimal psychological distress had elevated levels of cortisol at baseline and prolonged responses to stress. The results survived statistical control for participants' socioeconomic status, age, body mass index, smoking status, and current depression symptoms. There was also some evidence that this association was modified by gender, with the prolonged cortisol effect in the ELS/low-distress group more pronounced among women, and the blunted cortisol effect in the ELS/recurrent-distress group more pronounced among men.

Aim 2. Aims 2 and 3 were exploratory analyses. In Aim 2, my goals were to explore whether distinct clusters of stress-reactivity patterning existed within cortisol and the inflammatory protein IL-6, and if so, whether the clustering pattern within one biomarker was associated with clustering in the other. Using the mixed model approach of group-based trajectory modeling, I found that distinct clusters *did* appear to exist within both the cortisol and IL-6 data for this sample. The four fitted cortisol clusters were distinguished from each other by both mean baseline cortisol levels as well as slightly different reactivity patterns during the experimental laboratory session; the clusters corresponded to a "low-declining" group, a "middle-flat" group, a "higher-flat" group, and finally a "high-declining" group. Within the IL-6 data, the fitted solution comprised five trajectory groups, all of which shared a similar growth pattern (steadily increasing) but had slightly different mean baseline values. Results from my parametric bootstrap analysis, which evaluated the robustness of the model, were identical to those of the original fitted models. However, in a sensitivity analysis of the

parametric bootstrap procedure for the cortisol data, the model solutions were shown to be unstable, highlighting the non-robustness of the overall Nagin method.

Dual-membership probabilities for the various cortisol and IL-6 trajectory groups appeared to be moderately correlated with each other, such that "opposite" biomarker groups tended to associate together. For example, study participants who fell into the highest cortisol trajectory group also tended to fall into one of the lowest IL-6 trajectory groups, while participants falling into the lowest cortisol group had a higher probability of falling into a high IL-6 group. The principal finding from this "dual trajectory" analysis, however, was that membership in a given cortisol or IL-6 group is not a very sensitive predictor of group membership in the other biomarker.

Aim 3. For Aim 3, my goal was to explore whether early-life stress, longitudinal history of psychological distress, and other theoretically important variables predicted individuals' membership in the cortisol and IL-6 clusters. The results from this analysis were predominantly null with respect to ELS/distress status. Individuals exposed to ELS and who subsequent had recurring problems with depression and anxiety symptoms were marginally more likely to fall into the lowest cortisol trajectory group – a finding that is consistent with some of the piecewise growth-curve results from Aim 1 – but otherwise cortisol and IL-6 group membership was not significantly predicted by any combination of ELS exposure and psychological distress history. The timing of the participants' laboratory sessions was the strongest predictor of cortisol group membership, with afternoon-session participants far less likely to fall into the highest cortisol trajectory groups (no doubt due to the diurnal patterning of cortisol production). Age, body mass index, and civil service grade were the other significant predictors of both cortisol and IL-6 group memberships. Older participants were more likely, and participants with a high BMI less likely, to fall into the highest cortisol group. Older participants were also more likely to be in a high IL-6 group. However, increasing BMI meant a *decreasing* probability of falling into a low IL-6 group. For both biomarkers, participants of lower socioeconomic status (indexed by civil service grade) were less likely to fall into the lowest trajectory groups.

4.2 Contributions to the Existing Literature

This dissertation research builds on a large existing literature with similar aims – using psychobiological data to better understand the mechanisms that connect early-life adversity with later health problems – but contributes to and extends it in several notable respects. Most importantly, my research places this debate within a broadly epidemiological context, and seeks to inform the extent to which physiological stress reactivity patterns may underlie the *population* mental health differences observed between ELS-exposed and non-ELS exposed groups. A crucial aspect of this framework is the lifecourse approach outlined in the Introduction (Chapter 1). This lifecourse approach is exemplified by my use of longitudinal psychological data to characterize study participants' lifecourse experience of mental health problems in adulthood. Nearly all previous studies in this literature limit their examination of participants' psychological attributes to current symptoms, which may or may not reflect the broader trend of their lifetime mental health experiences. Since early-life adversity is known to predict mental health problems that are often unusually recurrent and severe [9, 11, 38, 57], the fact that my research explicitly examines the longitudinal patterning of individuals' psychological experiences is a valuable contribution. In another useful addition to the

epidemiological psychobiological literature, my study's findings also emerge from one of the largest and most socioeconomically diverse samples in which HPA axis and immune reactivity have been assessed in a controlled laboratory setting, increasing the generalizability of the results.

The divergent cortisol response patterns I observed in my Aim 1 findings are similar to those reported in one previous study that analyzed current psychological symptoms as a moderator of the ELS-HPA axis reactivity relationship [106]. These patterns, however, are different from those found in some other previous studies [23, 81, 97, 99, 100, 105, 180]. Those studies mostly reported that ELS-exposed people with no current psychiatric problems had blunted cortisol reactivity in comparison to non-ELS-exposed people. However, mixed or non-significant results have also been reported [99, 100, 104].

In my Aim 3 analyses, I did not find any relationship between joint ELS/psychological history status and immune reactivity. Although no other study has specifically examined IL-6 acute stress reactivity among participants classified according to both ELS exposure and psychological distress history, my exploratory results do contradict other related findings. Of most relevance are two studies that found, respectively, that psychiatrically healthy males and females with ELS had increased acute IL-6 responses to stress [116], and that depressed males with ELS had both increased basal and acute IL-6 responses [115]. There are also reports finding that basal inflammation in ELS-exposed people is much more pronounced among those who were depressed, and that levels of inflammation among ELS-exposed people who are *not* depressed are similar to those in people not exposed to ELS at all [108, 111].

Methodological differences may shed light on these inconsistencies. Most of the previous studies examining cortisol reactivity, with the obvious exception of Harkness et al. [106], did not stratify their ELS-exposed participants by psychological status. Although many such cortisol studies restricted their samples to only include ELS-exposed individuals who were currently psychologically healthy, those "healthy" ELS-exposed groups very often had significantly higher levels of depression symptomatology when compared to the control groups. These "healthy" ELS-exposed samples could therefore have included people who, while not currently meeting clinical criteria for depression, may still have had subclinical disorder. By not stratifying further on depression symptoms *among* the ELS-exposed groups, and in particular by not examining their long-term histories of symptoms, previous analyses may have missed additional heterogeneity that existed within these groups. With regard to IL-6, the literature is still too sparse to draw any conclusions about whether my findings change our inference about the pathways connecting ELS to psychological disorder. The Pace et al. [115] and Carpenter et al. [116] studies were very small, with sample sizes between 19 and 27 individuals, leaving their results necessarily very tentative. My sample size was far larger (n=543) and arguably more population-representative. The Danese et al. [108, 111] studies, on the other hand, utilized large, well-designed, population-representative samples, so their findings must be considered robust. However, these authors used a different measure of inflammation (C-reactive protein rather than IL-6), as well as different statistical methods, so the results are not truly comparable. In future analyses, I would like to use my study sample to assess whether differences in C-reactive protein are observed among ELS-exposed people with and without histories of recurrent psychological distress.

The inconsistency of my Aim 1 results with some other literature may also be partly explained by the age of my sample. Most prior studies assessing cortisol reactivity used adolescent or young-adult samples, compared to my sample of older adults. A meta-analysis

has found that as sample age increased, depressed individuals showed increasingly blunted cortisol stress responses [123]. Lastly, many cortisol studies used the dexamethasone suppression test (DST) rather than psychological challenge tests, leaving the comparability of their results uncertain [23]. Notably, my Aim 1 findings are consistent with literature showing that depression and anxiety symptoms are associated with blunted cortisol reactivity [123-127]. In my study, participants (particularly men) with a history of ELS and recurrent severe psychological distress – which may have qualified at one or more times as clinically significant – demonstrated this same cortisol response blunting. Dysregulation of the HPA axis in depression is believed to result, in part, from reduced feedback inhibition by endogenous glucocorticoids [77]. However, data on HPA axis functioning in depression are generally complex and often difficult to interpret, and observed effects can depend on numerous factors [181]. Future research should seek to replicate my results in a similarly older population, again using a psychological challenge test.

Lastly, my findings suggest that exposure to even just one type of early-life stress can have significant effects on the biology and mental health risk of those exposed. Nearly sixty percent of the ELS/low-distress group in my sample, and nearly fifty percent of the ELS/recurrent-distress group, reported experiencing only one kind of ELS category. While it is true that some of them may have been exposed to a category that was not assessed in the data collections, this statistic is still striking, especially given the significant differences in cortisol response patterns observed between the groups. This observation is consistent with other reports that, although increasing numbers of adversities are associated in a dose-response fashion with increased likelihood of psychological disorder, even individual adversities are associated with significantly increased risk (see Chapter 1 for a review). My study was not adequately powered to investigate whether specific types of adversity were especially powerful predictors of recurrent depression/anxiety symptoms, although statistically the ELS/low-distress and ELS/recurrent-distress groups only differed by the proportion reporting parental death. Future work is needed to explore this question more thoroughly.

4.3 Implications of the Findings

The inconsistency of the previous literature in this field did not encourage the formulation of specific hypotheses about the nature of the relationship between early-life stress, longitudinal psychological symptoms, and physiological stress reactivity. My test was therefore necessarily against the null, rather than against any particular theoretical prediction, and must be considered preliminary until further replications in other datasets can be done.

Implications for population health. If it is true that physiological reactivity patterns observed in late life are the same as those that would be observed in earlier life, then my findings are consistent with the supposition that two (or more) different patterns of atypical cortisol stress reactivity may be encoded in the aftermath of ELS, and in turn promote differential susceptibility to lifetime psychological disorder [72, 80]. In particular, a generous interpretation of my Aim 1 results would suggest that some of the increased longitudinal depression and anxiety symptoms observed in a subset of ELS-exposed populations may be due to blunted cortisol reactivity, while prolonged cortisol responses may account for the comparative psychological resiliency in another subset of the ELS-exposed population. This interpretation would further assert, given my Aim 3 findings, that this divergence in ELS-

exposed populations' longitudinal psychological symptoms cannot be explained by differences in inflammation, at least as measured by basal and stress-reactive levels of interleukin-6.

This generous interpretation might similarly shed interesting light on the question of how "adaptive" different cortisol patterns are. Because chronically high levels of cortisol suppress the immune system, permanently hyporeactive cortisol responses in ELS-exposed individuals might better maintain the immune system's ability to cope with pathogen invasion or wounds [182]. Yet this hyporeactivity may also induce inflammation-related diseases – including depression – or autoimmune disorders over time, since the immune system's reactions to pathogens or endogenous systemic inflammation would be unrestrained by cortisol's anti-inflammatory effects [72]. Recurrent depressive episodes alone might be detrimental to the organism: there is preliminary, although not conclusive, evidence that depression is associated with reproductive disadvantage [183]. It is somewhat more plausible that a hyperreactive stress reactivity phenotype would be adaptive for survival and reproduction. For example, increased reactivity enhances vigilance in the face of threat, reduces risk of sepsis upon later encounters with pathogens, and may reduce the likelihood of engaging in risky behaviors in low socioeconomic status, high-crime environments (see Zhang et al. [184] for a review). Conversely, hyperreactive phenotypes may lead to allostatic load, which increases risk for gastrointestinal ulceration, diabetes mellitus, immune system atrophy, and coronary artery disease [72].

I cannot at this point, however, draw any conclusions about the causal nature of my observations. The temporal ordering of my study variables (i.e., the fact that the physiological data were measured subsequent to the reported occurrence of ELS and psychological symptoms) prevents any causal interpretation of my findings. Future investigations may prove that physiological reactivity patterns are a stable trait characteristic over adulthood and that my findings are unlikely to be due to reverse causation. If this were true, I would more comfortably conclude that cortisol reactivity patterns play a causal role in leading to these diverging trajectories of psychological problems. With what we now know, however, I interpret my findings as a very provocative association that deserves further exploration in other samples that have access to longitudinal psychological data. In addition, unless one were prepared to conclude that recurrent depression symptoms are a reliable signal that cortisol hyporeactivity has lower adaptive value, while minimal depression signals the higher adaptability of cortisol hyperreactivity – which I am not prepared to do – then my study cannot inform the debate about the relative "adaptiveness" of these cortisol patterns. This is especially true because my sample is made up of unusually healthy older people. However, the sample will continue to be tracked as the Whitehall study continues into the future, and the relative adaptiveness of their cortisol patterns may become clearer as the sample ages and becomes ill over time.

Regardless of the temporal ordering and causality issues inherent in my study design, the group physiological reactivity differences I observed were small, and it is unclear how much of the burden of depression/anxiety symptoms in ELS-exposed populations they might explain. It remains unknown what magnitude of an alteration in cortisol reactivity or inflammation levels is necessary to induce psychological disorder, if indeed that relationship is causal. Meta-analyses and other reviews have suggested that group differences in basal and stress-reactive cortisol production are fairly small (0.5-3.0 nmol/L) in depressed vs. non-depressed samples, so a seemingly minor biological difference may in fact translate to a meaningful impact on health [123, 181, 185]. Similarly, IL-6 levels in depressed vs. non-depressed samples have

been found to differ on average by 1.78 pg/mL [186]. As these biomarkers necessarily operate within a narrow biological range, it might be expected that any group differences would be small, since gross deviations from that biological range could presumably lead to organismal failure. On the other hand, these small differences could also mean that cortisol (or inflammation) levels are not in fact a primary driver of depression/anxiety incidence. From an epidemiological perspective attempting to account for the "proportion of disease explained," other pathways between ELS and long-term psychological risk, such as harmful health behaviors or cognitive coping styles, may provide an equally or more significant contribution. I attempted to control for the effects of some of these potential mediators, but not all the relevant variables were available. Much more work in this area needs to be done.

Implications for population heterogeneity in cortisol and IL-6 stress responses. In my study sample of approximately 540 older adults, I found preliminary evidence that cortisol and IL-6 stress-response patterns cluster into distinct subgroups. Population heterogeneity in the level and shape of cortisol responses appears to be greater than that of IL-6 responses. Heterogeneity in IL-6 response clusters appeared to be driven primarily by variation around baseline values, while the cortisol clusters were defined both by trajectory shape as well as baseline values. As there is somewhat better theoretical rationale for the existence of distinct clusters of cortisol response shapes than there is for IL-6 clusters, this result was perhaps not too surprising [72, 87, 171, 172]. Although clustering methods like group-based trajectory modeling have been infrequently used to examine acute biomarker responses, my findings suggest that future psychobiological studies may profit from examining clustering in participants' cortisol and IL-6 responses using these methods. However, as noted frequently throughout this dissertation, such analyses are best suited for pattern detection purposes only, rather than for drawing firm inference from the results. Conducting sensitivity analyses of the type I performed for the cortisol data will give future researchers a sense of how stable (or unstable) their results are.

Unfortunately, my study cannot shed light on *why* these different patterns of atypical cortisol and IL-6 reactivity develop in a population in the first place. The most commonly cited hypothesis is that genetic predispositions interact with the experience of early-life adversity and give rise to these different phenotypes. Several genetic polymorphisms (particularly the serotonin transporter gene promoter, 5-HTTLPR) appear to moderate vulnerability to major depression following adverse early experiences [37, 187]. Animal models [86, 188] and some human data [37, 189] suggest that such gene-environment interactions may operate through the developmental programming of gene expression and epigenetic modifications in both brain and peripheral tissues [190]. The physiological processes regulated by these genetic loci are often closely intertwined with HPA axis functioning [191-193], and the effects of one or more such polymorphisms may help explain my findings.

Implications for policymaking. Many researchers in the field of developmental psychobiology are interested in the biological pathways connecting ELS to mental health risk because they believe that such research may eventually lead to therapeutic approaches (pharmacological or otherwise) that can alleviate suffering in people exposed to ELS. As a researcher trained in the public health tradition, I remain skeptical of the utility of individual therapeutics for an extraordinarily common exposure like early-life adversity. Primary prevention may be a far more effective approach, especially since ELS is associated not only with mental health problems but also with a host of other detrimental social and behavioral

sequelae that may not be reduced by individual therapeutics. The total effect of ELS on the population – not just on those directly exposed, but in the "ripple effect" these sequelae have on the other family members, friends, romantic partners, and children of the exposed – is probably great. Although I have little experience with policymaking, my hope for this research is that highlighting the potential *biological* effects of ELS and their role in giving rise to observable psychological sequelae may help convince policymakers that early-life adversity is a critical social and moral concern, and that we as a society should devote resources to preventing its occurrence.

4.4 Methodological Considerations

My study has limitations. As noted above, temporality of the relationship between cortisol and IL-6 reactivity patterns and history of psychological symptoms cannot be established using this dataset. As with all studies in this field, multiple lifelong prospective assessments of physiological reactivity and psychological symptoms among ELS-exposed subjects would be necessary to demonstrate a causal relationship. However, my analysis provides a unique contribution through its use of a large study population and a longitudinal, prospective measure of psychological distress symptoms, measured over nearly twenty years. Prospective ascertainment of psychological symptoms is substantially more reliable than retrospective recalls [194].

Limitations of the measures. On a related note, I (like other researchers working in this field) conceptualized the later-life measures of cortisol and IL-6 reactivity as proxies for those in earlier life, based on several methodological studies have provided support for the supposition that physiological reactivity is a stable trait [195-198]. Needless to say, further investigation of this assumption is warranted. It is also possible that another, unknown factor is responsible for both psychological vulnerability and physiological reactivity pattern. For example, there is some evidence that chronic exposure to stressful events is associated with reduced physiological reactivity to new stressors over time [182, 199, 200], a hypothesis that I was unable to examine in this study.

All self-reported measures of ELS are subject to bias, and the measures used in this study are relatively crude. Studies examining ELS more typically use measures such as the Childhood Trauma Questionnaire [201], which are generally validated according to their reliability and less typically for their actual validity, since documentation of traumatic events is so difficult [202]. However, there is evidence that the magnitude of bias resulting from the use of *any* ELS measure is often modest, that ELS is more likely to be underreported than overreported, and that basic questions pertaining to serious, readily operationalized experiences – such as those used in the Whitehall and HSS protocols – are more reliable than detailed queries [53, 203-205].

I also was not able to use information on clinical psychiatric diagnoses or treatments in my measure of psychological history, as that information was not collected in the Whitehall II study. However, there are several reasons that this may not be a large concern. First, the GHQ-28 is a valid measure of severe psychological distress, and can function as a proxy for likely clinical disorder [148-150]. Second, recent research has found that the observed effect of childhood maltreatment on later psychiatric problems may primarily be due to non-specific latent liabilities for internalizing and externalizing psychopathological symptoms, rather than specific psychiatric diagnoses – thus, the GHQ may capture this effect as well as would

diagnostic evaluations [206]. Third, psychological distress is a legitimate concern in its own right, both as an important source of population morbidity [26, 27] and as a risk factor for coronary heart disease [28] and other outcomes.

Lastly, selection criteria for HSS participants limits my study's generalizability in terms of the original Whitehall II cohort and its British source population. Individuals exposed to ELS who had severe mental and physical disorders in 2008 would have been excluded from the HSS sample, resulting in an atypically resilient study population. However, the HSS sample was similar to the overall baseline WH sample on many characteristics [140], and their healthiness means that my findings cannot be attributed to underlying disease. Furthermore, their Whitehall GHQ scores indicate that a substantial proportion of them had significant prior psychological problems, despite the exclusion criteria. As with any study, replication will be necessary; until then my findings should be interpreted with caution.

Limitations of the statistical methods. As described in the Methods section (Chapter 2), both statistical approaches used in this dissertation have fundamental weaknesses, largely stemming from the untestable assumptions that the models make and their parametric structure. In the case of random-effects growth curve modeling, the model assumes that the random regression coefficients have a bivariate normal distribution with mean zero and unstructured covariance matrices. In the case of group-based trajectory modeling (GBTM), the technique assumes that the residual error distributions are independent and normally distributed within the identified trajectory groups [173]. This last assumption necessitates the log transformation of skewed data such as cortisol and IL-6 values, which may artificially condense the data and influence the cluster findings. Furthermore, model selection in GBTM is not automated and is subject to user-defined heuristic judgments, which negates the generalizability of the results across samples and populations. Statistical methods for cluster analysis are in general prone to this problem – even when drawn from the same source population, different samples may give rise to different clustering solutions.

For both of my analyses, I attempted to evaluate the effect of these assumptions on my results through the use of bootstrapping. For my Aim 1 analyses, in which I used nonparametric bootstrapping, the inference was very similar, and I therefore concluded that the random effects model's assumptions were not badly violated. For my Aim 2 and 3 analyses, in which I used parametric bootstrapping, the bootstrapping approach does not actually test the model – it assumes the model is correct, even when this assumption is very unlikely. The sensitivity analyses gave a preliminary indication of the instability of the group-based trajectory modeling approach. Future work in this field that uses either random-effects growth curve modeling or GBTM should also employ appropriate bootstrapping and sensitivity analysis techniques, to ensure that the informal inference on observed results is not over-interpreted.

4.5 Future Directions

The research undertaken for my dissertation has provided a novel epidemiologic contribution to the field of developmental psychobiology, and cast some light onto the physio-logical processes that may connect early-life adversity to later-life mental health risk. Yet there is a tremendous amount of work still to be done in order to fully elucidate these pathways.

First, as noted above, replication of my research questions should be attempted in other samples that also have longitudinal psychological data. Replication would address the question

of whether the interaction effect I observed between ELS, longitudinal psychological distress, and cortisol reactivity patterns is generalizable to other populations across time, location, and sociodemographic makeup. It would also allow for exploration of the consistency of clustering patterns in stress-reactive cortisol and IL-6 levels. Although cluster results are inherently unstable when comparing different populations, if biological reactivity profiles do in fact reflect reliable information about the underlying functioning of those systems, then the overall patterns of response observed may be similar across populations even if the proportion of individuals falling into each cluster changes.

Additionally, replication in other (and hopefully larger) samples would permit further examination of whether the observed effects are modified by gender or type of early-life stress. As I reported preliminary evidence of moderation by gender of the effect of early-life stress and psychological distress on cortisol reactivity, future work should explore whether this effect is real, and what might be driving it. Similarly, it would be fairly logical to conclude that certain types of early-life stress – e.g., parental divorce or death – will have different effects on the child depending on extenuating circumstances. For example, a parent's death could have very different sequelae depending on the coping ability of the surviving parent, the availability of other supportive family members or friends, the age of the child at the time of the death, etc. In contrast, physical abuse is likely to have a more consistently detrimental effect on the child. The nuances of these differences in ELS exposure type are an area ripe for further research. I think it would be particularly valuable to combine such epidemiological and biological data with detailed "personal history" data derived from interviews. The richness of detail about the circumstances of the childhood adversity, obtainable only from such in-depth interviews, might shed light on individual biological differences as well as elicit new lines of inquiry.

As mentioned previously, the fundamental question of *why* different patterns of atypical cortisol and IL-6 reactivity develop in a population the first place remains to be answered. Currently, the most workable hypothesis is that genetic variants are at least partly responsible. Logistically, however, datasets that can be used to address this question – i.e., have all the necessary variables – are very rare. Yet this is a vital question, since only through establishing this fundamental cause will researchers be able to address the "chicken-or-the-egg" problem that plagues this field of inquiry. There is a good chance that genetic data will become available for the dataset I used for this dissertation, so in future work I may be able to start investigating whether specific genetic variants drive the association between ELS, lifetime psychological distress, and biological reactivity. I also note that plasticity of the kind described by the developmental programming literature is not the only explanation for observable biological characteristics of a given population. Maternal experiences that occur post-conception but prior to birth may have a substantial selection effect on a cohort of infants in gestation, such that those infants surviving to birth are a non-random sample with distinctive biological traits [207, 208]. Thus, both selection and plasticity may shape the hormonal and immunological functioning of living humans.

Overall, this dissertation research demonstrates how a lifecourse epidemiologic framework can lead to novel questions and rigorous analyses designed to inform how social experiences "get under the skin" and contribute to population ill-health. It is my hope that this work will lead to a better understanding of the biological sequelae of early-life adversity and the effects those sequelae can have on psychological risk and resilience throughout the lifecourse.

REFERENCES

1. Kuh D. and Ben-Shlomo Y., eds. *A life course approach to chronic disease epidemiology*. Second ed. 2004, Oxford University Press: Oxford.
2. Barker D.J. Fetal origins of coronary heart disease. *BMJ* 1995; **311**(6998): p. 171-4.
3. Barker D.J. In utero programming of chronic disease. *Clinical Science* 1998; **95**(2): p. 115-28.
4. Kessler R.C., Davis C.G., and Kendler K.S. Childhood adversity and adult psychiatric disorder in the US National Comorbidity Survey. *Psychological Medicine* 1997; **27**: p. 1101-1119.
5. Kessler R.C. and Magee W.J. Childhood adversities and adult depression: Basic patterns of association in a United States national survey. *Psychological Medicine* 1993; **23**(3): p. 679-690.
6. Felitti V.J., Anda R.F., Nordenberg D., Williamson D.F., et al. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults - The adverse childhood experiences (ACE) study. *American Journal of Preventive Medicine* 1998; **14**(4): p. 245-258.
7. Heim C., Plotsky P.M., and Nemeroff C.B. Importance of studying the contributions of early adverse experience to neurobiological findings in depression. *Neuropsychopharmacology* 2004; **29**(4): p. 641-648.
8. Cohen R.A., Paul R.H., Stroud L.R., Gunstad J., et al. Early life stress and adult emotional experience: An international perspective. *International Journal of Psychiatry in Medicine* 2006; **36**(1): p. 35-52.
9. Widom C.S., DuMont K., and Czaja S.J. A prospective investigation of major depressive disorder and comorbidity in abused and neglected children grown up. *Archives of General Psychiatry* 2007; **64**(1): p. 49-56.
10. Mersky J.P. and Topitzes J. Comparing early adult outcomes of maltreated and non-maltreated children: A prospective longitudinal investigation. *Children and Youth Services Review* 2009; **32**(8): p. 1086-1096.
11. Wiersma J.E., Hovens J., van Oppen P., Giltay E.J., et al. The importance of childhood trauma and childhood life events for chronicity of depression in adults. *Journal of Clinical Psychiatry* 2009; **70**(7): p. 983-989.
12. Slopen N., Williams D.R., Seedat S., Moomal H., et al. Adversities in childhood and adult psychopathology in the South Africa Stress and Health Study: Associations with first-onset DSM-IV disorders. *Social Science & Medicine* 2010; **71**(10): p. 1847-1854.
13. Dong M.X., Giles W.H., Felitti V.J., Dube S.R., et al. Insights into causal pathways for ischemic heart disease - Adverse childhood experiences study. *Circulation* 2004; **110**(13): p. 1761-1766.
14. Dube S.R., Fairweather D., Pearson W.S., Felitti V.J., et al. Cumulative childhood stress and autoimmune diseases in adults. *Psychosomatic Medicine* 2009; **71**(2): p. 243-250.
15. McCrory E., De Brito S.A., and Viding E. Research Review: The neurobiology and genetics of maltreatment and adversity. *Journal of Child Psychology and Psychiatry* 2010; **51**(10): p. 1079-1095.

16. Taylor S.E. Mechanisms linking early life stress to adult health outcomes. *Proceedings of the National Academy of Sciences of the United States of America* 2010; **107**(19): p. 8507-8512.

17. Miller G.E., Chen E., and Parker K.J. Psychological stress in childhood and susceptibility to the chronic diseases of aging: Moving toward a model of behavioral and biological mechanisms. *Psychological Bulletin* 2011; **137**(6): p. 959-997.

18. Silk J.S., Vanderbilt-Adriance E., Shaw D.S., Forbes E.E., et al. Resilience among children and adolescents at risk for depression: Mediation and moderation across social and neurobiological contexts. *Development and Psychopathology* 2007; **19**(3): p. 841-865.

19. Cohen S., Kessler R.C., and Underwood L.G. *Strategies for measuring stress in studies of psychiatric and physical disorders*, in *Measuring stress: A guide for health and social scientists*. S. Cohen, R.C. Kessler, and L.G. Underwood, Editors. 1995, Oxford University Press: New York, NY.

20. Hales C.N. and Barker D.J. The thrifty phenotype hypothesis. *British Medical Bulletin* 2001; **60**: p. 5-20.

21. Wadhwa P.D. Psychoneuroendocrine processes in human pregnancy influence fetal development and health. *Psychoneuroendocrinology* 2005; **30**(8): p. 724-43.

22. Taylor S.E., Lerner J.S., Sage R.M., Lehman B.J., et al. Early environment, emotions, responses to stress, and health. *Journal of Personality* 2004; **72**(6): p. 1365-1393.

23. Rao U., Hammen C., Ortiz L.R., Chen L.A., et al. Effects of early and recent adverse experiences on adrenal response to psychosocial stress in depressed adolescents. *Biological Psychiatry* 2008; **64**(6): p. 521-526.

24. Repetti R.L., Taylor S.E., and Seeman T.E. Risky families: family social environments and the mental and physical health of offspring. *Psychological Bulletin* 2002; **128**(2): p. 330-66.

25. Cicchetti D. and Blender J.A. A multiple-levels-of-analysis perspective on resilience: implications for the developing brain, neural plasticity, and preventive interventions. *Ann N Y Acad Sci* 2006; **1094**: p. 248-58.

26. Dohrenwend B.P., Shrout P.E., Egri G., and Mendelsohn F.S. Nonspecific psychological distress and other dimensions of psychopathology. *Arch Gen Psychiatry* 1980; **37**: p. 1229-123.

27. Broadhead W.E., Blazer D.G., George L.K., and Tse C.K. Depression, disability days, and days lost from work in a prospective epidemiologic survey. *JAMA* 1990; **264**(19): p. 2524-8.

28. Stansfeld S.A., Fuhrer R., Shipley M.J., and Marmot M.G. Psychological distress as a risk factor for coronary heart disease in the Whitehall II Study. *International Journal of Epidemiology* 2002; **31**(1): p. 248-255.

29. Luthar S.S., Cicchetti D., and Becker B. The construct of resilience: A critical evaluation and guidelines for future work. *Child Development* 2000; **71**(3): p. 543-562.

30. Vanderbilt-Adriance E. and Shaw D.S. Conceptualizing and re-evaluating resilience across levels of risk, time, and domains of competence. *Clin Child Fam Psychol Rev* 2008; **11**(1-2): p. 30-58.

31. Luthar S.S. and Zelazo L.B. *Research on resilience: An integrative review*, in *Resilience and vulnerabilty: Adaptation in the context of childhood adversities*. S.S. Luthar, Editor 2003, Cambridge University Press: New York, NY.

32.   Tough P. The poverty clinic. *The New Yorker*, March 11, 2011, 25-32.
33.   Green J.G., McLaughlin K.A., Berglund P., Gruber M., et al. Childhood adversities and adult psychiatric disorders in the National Comorbidity Survey Replication I *Archives of General Psychiatry* 2010; **67**(2): p. 113-123.
34.   Menard C., Bandeen-Roche K.J., and Chilcoat H.D. Epidemiology of multiple childhood traumatic events: child abuse, parental psychopathology, and other family-level stressors. *Social Psychiatry and Psychiatric Epidemiology* 2004; **39**(11): p. 857-865.
35.   Scher C.D., Forde D.R., McQuaid J.R., and Stein M.B. Prevalence and demographic correlates of childhood maltreatment in an adult community sample. *Child Abuse & Neglect* 2004; **28**(2): p. 167-180.
36.   Kessler R.C., McLaughlin K.A., Green J.G., Gruber M.J., et al. Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. *British Journal of Psychiatry* 2010; **197**(5): p. 378-85.
37.   Polanczyk G., Caspi A., Williams B., Price T.S., et al. Protective effect of CRHR1 gene variants on the development of adult depression following childhood maltreatment: replication and extension. *Archives of General Psychiatry* 2009; **66**(9): p. 978-85.
38.   Felitti V.J. and Anda R.F. *The relationship of adverse childhood experiences to adult medical disease, psychiatric disorders, and sexual behavior: Implications for healthcare*, in *The Hidden Epidemic: The Impact of Early Life Trauma on Health and Disease*. E. Vermetten and R. Lanius, Editors. 2009, Cambridge University Press: Cambridge.
39.   Breslau N., Kessler R.C., Chilcoat H.D., Schultz L.R., et al. Trauma and posttraumatic stress disorder in the community: the 1996 Detroit Area Survey of Trauma. *Archives of General Psychiatry* 1998; **55**: p. 626-632.
40.   Edwards V., Holden G.W., Felitti V.J., and Anda R.F. Relationship between multiple forms of childhood maltreatment and adult mental health in community respondents: results from the adverse childhood experiences study. *American Journal of Psychiatry* 2003; **160**(8): p. 1453-60.
41.   Dong M., Anda R.F., Felitti V.J., Dube S.R., et al. The interrelatedness of multiple forms of childhood abuse, neglect, and household dysfunction. *Child Abuse & Neglect* 2004; **28**(7): p. 771-84.
42.   Kessler R.C. The global burden of anxiety and mood disorders: Putting the European Study of the Epidemiology of Mental Disorders (ESEMeD) findings into perspective. *Journal of Clinical Psychiatry* 2007; **68**: p. 10-19.
43.   Kessler R.C., Chiu W.T., Demler O., and Walters E.E. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry* 2005; **62**(6): p. 617-627.
44.   Kessler R.C., Berglund P., Demler O., Jin R., et al. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry* 2005; **62**(6): p. 593-602.
45.   Solomon D.A., Keller M.B., Leon A.C., Mueller T.I., et al. Multiple recurrences of major depressive disorder. *American Journal of Psychiatry* 2000; **157**(2): p. 229-33.
46.   Kessler R.C., Avenevoli S., Green J., Gruber M.J., et al. National Comorbidity Survey Replication Adolescent Supplement (NCS-A): III. Concordance of DSM-IV/CIDI

diagnoses with clinical reassessments. *Journal of the American Academy of Child and Adolescent Psychiatry* 2009; **48**(4): p. 386-399.

47. Michaud C.M., Murray C.J.L., and Bloom B.R. Burden of disease - Implications for future research. *JAMA: The Journal of the American Medical Association* 2001; **285**(5): p. 535-539.

48. Revicki D.A., Travers K., Wyrwich K.W., Svedsater H., et al. Humanistic and economic burden of generalized anxiety disorder in North America and Europe. *Journal of Affective Disorders* 2011.

49. Simon G.E. Social and economic burden of mood disorders. *Biological Psychiatry* 2003; **54**(3): p. 208-15.

50. Olesen J., Gustavsson A., Svensson M., Wittchen H.U., et al. The economic cost of brain disorders in Europe. *European Journal of Neurology* 2012; **19**(1): p. 155-162.

51. Culpepper L. Understanding the burden of depression. *Journal of Clinical Psychiatry* 2011; **72**(6): p. e19.

52. Knudsen E.I., Heckman J.J., Cameron J.L., and Shonkoff J.P. Economic, neurobiological, and behavioral perspectives on building America's future workforce. *Proceedings of the National Academy of Sciences of the United States of America* 2006; **103**(27): p. 10155-10162.

53. Anda R.F., Felitti V.J., Bremner J.D., Walker J.D., et al. The enduring effects of abuse and related adverse experiences in childhood - A convergence of evidence from neurobiology and epidemiology. *European Archives of Psychiatry and Clinical Neuroscience* 2006; **256**(3): p. 174-186.

54. Tonmyr L., Williams G., Hovdestad W.E., and Draca J. Anxiety and/or depression in 10-15-year-olds investigated by child welfare in Canada. *Journal of Adolescent Health* 2011; **48**(5): p. 493-498.

55. Comijs H.C., Beekman A.T.F., Smit F., Bremmer M., et al. Childhood adversity, recent life events and depression in late life. *Journal of Affective Disorders* 2007; **103**(1-3): p. 243-246.

56. Korkeila J., Vahtera J., Nabi H., Kivimaki M., et al. Childhood adversities, adulthood life events and depression. *Journal of Affective Disorders* 2010; **127**(1): p. 130-138.

57. Nanni V., Uher R., and Danese A. Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: A meta-analysis. *American Journal of Psychiatry* 2012; **169**(2): p. 141-151.

58. Dube S.R., Anda R.F., Felitti V.J., Chapman D.P., et al. Childhood abuse, household dysfunction, and the risk of attempted suicide throughout the life span: findings from the Adverse Childhood Experiences Study. *JAMA* 2001; **286**(24): p. 3089-96.

59. Raikkonen K. and Pesonen A.K. Early life origins of psychological development and mental health. *Scandinavian Journal of Psychology* 2009; **50**(6): p. 583-591.

60. Kiecolt-Glaser J.K., Gouin J.P., Weng N.P., Malarkey W.B., et al. Childhood adversity heightens the impact of later-life caregiving stress on telomere length and inflammation. *Psychosomatic Medicine* 2011; **73**(1): p. 16-22.

61. Gunnar M. and Quevedo K. The neurobiology of stress and development. *Annual Review of Psychology* 2007; **58**: p. 145-173.

62. Eisenberg N., Spinrad T.L., and Eggum N.D. Emotion-related self-regulation and its relation to children's maladjustment. *Annual Review of Clinical Psychology* 2010; **6**: p. 495-525.

63. Jackowski A., Perera T.D., Abdallah C.G., Garrido G., et al. Early-life stress, corpus callosum development, hippocampal volumetrics, and anxious behavior in male nonhuman primates. *Psychiatry Research-Neuroimaging* 2011; **192**(1): p. 37-44.

64. Pechtel P. and Pizzagalli D.A. Effects of early life stress on cognitive and affective function: an integrated review of human literature. *Psychopharmacology* 2011; **214**(1): p. 55-70.

65. McEwen B.S. Effects of adverse experiences for brain structure and function. *Biological Psychiatry* 2000; **48**(8): p. 721-31.

66. Cacioppo J.T., Berntson G.G., Malarkey W.B., Kiecolt-Glaser J.K., et al. Autonomic, neuroendocrine, and immune responses to psychological stress: the reactivity hypothesis. *Annals of the New York Academy of Sciences* 1998; **840**: p. 664-73.

67. Steptoe A., Hamer M., and Chida Y. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain, behavior, and immunity* 2007; **21**(7): p. 901-12.

68. Moberg G.P. and Mench J.A., eds. *The biology of animal stress: Basic principles and implications for animal welfare*. 2000, CABI Publishing: Oxon, UK.

69. Cannon W.B. *Bodily changes in pain, hunger, fear, and rage*. 1929, New York: Appleton-Century-Crofts.

70. Black P. Stress and the inflammatory response: A review of neurogenic inflammation. *Brain Behav Immun* 2002; **16**: p. 622-653.

71. Sapolsky R.M., Romero L.M., and Munck A.U. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* 2000; **21**(1): p. 55-89.

72. McEwen B.S. and Seeman T. Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. *Annals of the New York Academy of Sciences* 1999; **896**: p. 30-47.

73. McEwen B.S. and Stellar E. Stress and the individual. Mechanisms leading to disease. *Archives of Internal Medicine* 1993; **153**(18): p. 2093-101.

74. Streeck-Fischer A. and van der Kolk B.A. Down will come baby, cradle and all: diagnostic and therapeutic implications of chronic trauma on child development. *Australian and New Zealand Journal of Psychiatry* 2000; **34**(6): p. 903-918.

75. Luecken L.J. and Lemery K.S. Early caregiving and physiological stress responses. *Clinical Psychology Review* 2004; **24**(2): p. 171-191.

76. Tollenaar M.S., Beijers R., Jansen J., Riksen-Walraven J.M., et al. Maternal prenatal stress and cortisol reactivity to stressors in human infants. *Stress* 2011; **14**(1): p. 53-65.

77. Raison C.L. and Miller A.H. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. *American Journal of Psychiatry* 2003; **160**(9): p. 1554-65.

78. Finch C.E. and Crimmins E.M. Inflammatory exposure and historical changes in human life-spans. *Science* 2004; **305**(5691): p. 1736-1739.

79. De Bellis M.D. Developmental traumatology: The psychobiological development of maltreated children and its implications for research, treatment, and policy. *Development and Psychopathology* 2001; **13**(3): p. 539-564.

80. Boyce W.T. and Ellis B.J. Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Development and Psychopathology* 2005; **17**(2): p. 271-301.

81. Carpenter L.L., Carvalho J.P., Tyrka A.R., Wier L.M., et al. Decreased adrenocorticotropic hormone and cortisol responses to stress in healthy adults reporting significant childhood maltreatment. *Biological Psychiatry* 2007; **62**(10): p. 1080-1087.

82. Miller G.E., Chen E., and Zhou E.S. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin* 2007; **133**(1): p. 25-45.

83. Gunnar M.R., Frenn K., Wewerka S.S., and Van Ryzin M.J. Moderate versus severe early life stress: Associations with stress reactivity and regulation in 10-12-year-old children. *Psychoneuroendocrinology* 2009; **34**(1): p. 62-75.

84. Liu D., Diorio J., Tannenbaum B., Caldji C., et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* 1997; **277**(5332): p. 1659-62.

85. Caldji C., Tannenbaum B., Sharma S., Francis D., et al. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proceedings of the National Academy of Sciences U S A* 1998; **95**(9): p. 5335-40.

86. Szyf M., Weaver I.C., Champagne F.A., Diorio J., et al. Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Frontiers in Neuroendocrinology* 2005; **26**(3-4): p. 139-62.

87. Sanchez M.M., Ladd C.O., and Plotsky P.M. Early adverse experience as a developmental risk factor for later psychopathology: Evidence from rodent and primate models. *Development and Psychopathology* 2001; **13**(3): p. 419-449.

88. Weaver I.C.G., Cervoni N., Champagne F.A., D'Alessio A.C., et al. Epigenetic programming by maternal behavior. *Nature Neuroscience* 2004; **7**(8): p. 847-854.

89. Shannon C., Champoux M., and Suomi S.J. Rearing condition and plasma cortisol in rhesus monkey infants. *American Journal of Primatology* 1998; **46**(4): p. 311-321.

90. Lyons D.M., Yang C., Mobley B.W., Nickerson J.T., et al. Early environmental regulation of glucocorticoid feedback sensitivity in young adult monkeys. *Journal of Neuroendocrinology* 2000; **12**(8): p. 723-728.

91. Capitanio J.P., Mendoza S.P., Mason W.A., and Maninger N. Rearing environment and hypothalamic-pituitary-adrenal regulation in young rhesus monkeys (Macaca mulatta). *Developmental Psychobiology* 2005; **46**(4): p. 318-330.

92. Hart J., Gunnar M., and Cicchetti D. Salivary cortisol in maltreated children: Evidence of relations between neuroendocrince activity and social competence. *Development and Psychopathology* 1995; **7**(1): p. 11-26.

93. Fernald L.C.H., Burke H.M., and Gunnar M.R. Salivary cortisol levels in children of low-income women with high depressive symptomatology. *Development and Psychopathology* 2008; **20**(2): p. 423-436.

94. Fisher P.A., Kim H.K., Bruce J., and Pears K.C. Cumulative effects of prenatal substance exposure and early adversity on foster children's HPA-axis reactivity during a psychosocial stressor. *International Journal of Behavioral Development* 2012; **36**(1): p. 29-35.

95. Sturge-Apple M.L., Davies P.T., Cicchetti D., and Manning L.G. Interparental violence, maternal emotional unavailability and children's cortisol functioning in family contexts. *Developmental Psychology* 2012; **48**(1): p. 237-249.

96.   Ouellet-Morin I., Boivin M., Dionne G., Lupien S.J., et al. Variations in heritability of cortisol reactivity to stress as a function of early familial adversity among 19-month-old twins. *Archives of General Psychiatry* 2008; **65**(2): p. 211-218.

97.   MacMillan H.L., Georgiades K., Duku E.K., Shea A., et al. Cortisol response to stress in female youths exposed to childhood maltreatment: Results of the Youth Mood Project. *Biological Psychiatry* 2009; **66**(1): p. 62-68.

98.   Gordis E.B., Granger D.A., Susman E.J., and Trickett P.K. Salivary alpha amylase-cortisol asymmetry in maltreated youth. *Hormones and Behavior* 2008; **53**(1): p. 96-103.

99.   Heim C., Newport D.J., Heit S., Graham Y.P., et al. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA* 2000; **284**(5): p. 592-7.

100.   Heim C., Mletzko T., Purselle D., Musselman D.L., et al. The dexamethasone/corticotropin-releasing factor test in men with major depression: Role of childhood trauma. *Biological Psychiatry* 2008; **63**(4): p. 398-405.

101.   Girdler S.S., Sherwood A., Hinderliter A.L., Leserman J., et al. Biological correlates of abuse in women with premenstrual dysphoric disorder and healthy controls. *Psychosomatic Medicine* 2003; **65**(5): p. 849-856.

102.   Elzinga B.M., Roelofs K., Tollenaar M.S., Bakvis P., et al. Diminished cortisol responses to psychosocial stress associated with lifetime adverse events - A study among healthy young subjects. *Psychoneuroendocrinology* 2008; **33**(2): p. 227-237.

103.   Engert V., Efanov S.I., Dedovic K., Duchesne A., et al. Perceived early-life maternal care and the cortisol response to repeated psychosocial stress. *Journal of Psychiatry & Neuroscience* 2010; **35**(6): p. 370-377.

104.   Heim C., Newport D.J., Bonsall R., Miller A.H., et al. Altered pituitary-adrenal axis responses to provocative challenge tests in adult survivors of childhood abuse. *American Journal of Psychiatry* 2001; **158**(4): p. 575-581.

105.   Carpenter L.L., Tyrka A.R., Ross N.S., Khoury L., et al. Effect of childhood emotional abuse and age on cortisol responsivity in adulthood. *Biological Psychiatry* 2009; **66**(1): p. 69-75.

106.   Harkness K.L., Stewart J.G., and Wynne-Edwards K.E. Cortisol reactivity to social stress in adolescents: Role of depression severity and child maltreatment. *Psychoneuroendocrinology* 2011; **36**(2): p. 173-181.

107.   Danese A., Pariante C.M., Caspi A., Taylor A., et al. Childhood maltreatment predicts adult inflammation in a life-course study. *Proceedings of the National Academy of Sciences* 2007; **104**(4): p. 1319-1324.

108.   Danese A., Moffitt T.E., Pariente C.M., Ambler A., et al. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. *Archives of General Psychiatry* 2008; **65**(4): p. 409-416.

109.   Danese A., Moffitt T.E., Harrington H., Milne B.J., et al. Adverse childhood experiences and adult risk factors for age-related disease, depression, inflammation, and clustering of metabolic risk markers. *Archives of Pediatrics & Adolescent Medicine* 2009; **163**(12): p. 1135-1143.

110.   Slopen N., Lewis T.T., Gruenewald T.L., Mujahid M.S., et al. Early life adversity and inflammation in African Americans and whites in the Midlife in the United States Survey. *Psychosomatic Medicine* 2010; **72**(7): p. 694-701.

111. Danese A., Caspi A., Williams B., Ambler A., et al. Biological embedding of stress through inflammation processes in childhood. *Molecular Psychiatry* 2011; **16**(3): p. 244-246.

112. Wright R.J., Visness C.M., Calatroni A., Grayson M.H., et al. Prenatal maternal stress and cord blood innate and adaptive cytokine responses in an inner-city cohort. *American Journal of Respiratory and Critical Care Medicine* 2010; **182**(1): p. 25-33.

113. Carroll J.E., Cohen S., and Marsland A.L. Early childhood socioeconomic status is associated with circulating interleukin-6 among mid-life adults. *Brain Behavior and Immunity* 2011; **25**(7): p. 1468-1474.

114. Miller G.E., Chen E., Fok A.K., Walker H., et al. Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. *Proceedings of the National Academy of Sciences of the United States of America* 2009; **106**(34): p. 14716-14721.

115. Pace T.W.W., Mletzko T.C., Alagbe O., Musselman D.L., et al. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *American Journal of Psychiatry* 2006; **163**(9): p. 1630-U1.

116. Carpenter L.L., Gawuga C.E., Tyrka A.R., Lee J.K., et al. Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. *Neuropsychopharmacology* 2010; **35**(13): p. 2617-2623.

117. Miller G.E. and Chen E. Harsh family climate in early life presages the emergence of a proinflammatory phenotype in adolescence. *Psychological Science* 2010; **21**(6): p. 848-856.

118. Gold P.W., Goodwin F.K., and Chrousos G.P. Clinical and biochemical manifestations of depression - Relation to the neurobiology of stress, part 1. *New England Journal of Medicine* 1988; **319**(6): p. 348-353.

119. Gold P.W., Goodwin F.K., and Chrousos G.P. Clinical and biochemical manifestations of depression - Relation to the neurobiology of stress, part 2. *New England Journal of Medicine* 1988; **319**(7): p. 413-420.

120. Maes M., Yirmyia R., Noraberg J., Brene S., et al. The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metabolic Brain Disease* 2009; **24**(1): p. 27-53.

121. Raison C.L., Capuron L., and Miller A.H. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends in Immunology* 2006; **27**(1): p. 24-31.

122. Pariante C.M. and Lightman S.L. The HPA axis in major depression: classical theories and new developments. *Trends in Neurosciences* 2008; **31**(9): p. 464-468.

123. Burke H.M., Davis M.C., Otte C., and Mohr D.C. Depression and cortisol responses to psychological stress: A meta-analysis. *Psychoneuroendocrinology* 2005; **30**(9): p. 846-856.

124. Burke H.M., Fernald L.C., Gertler P.J., and Adler N.E. Depressive symptoms are associated with blunted cortisol stress responses in very low-income women. *Psychosomatic Medicine* 2005; **67**(2): p. 211-216.

125. Brooks K.P. and Robles T.F. Recent depressive and anxious symptoms predict cortisol responses to stress in men. *Psychoneuroendocrinology* 2009; **34**(7): p. 1041-1049.

126. Ahrens T., Deuschle M., Krumm B., van der Pompe G., et al. Pituitary-adrenal and sympathetic nervous system responses to stress in women remitted from recurrent major depression. *Psychosomatic Medicine* 2008; **70**(4): p. 461-467.

127. de Rooij S.R., Schene A.H., Phillips D.I., and Roseboom T.J. Depression and anxiety: Associations with biological and perceived stress reactivity to a psychological stress protocol in a middle-aged population. *Psychoneuroendocrinology* 2010; **35**(6): p. 866-877.

128. Schiepers O.J.G., Wichers M.C., and Maes M. Cytokines and major depression. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 2005; **29**(2): p. 201-217.

129. Glassman A.H. and Miller G.E. Where there is depression, there is inflammation... sometimes! *Biological Psychiatry* 2007; **62**(4): p. 280-281.

130. Dhabhar F.S., Burke H.M., Epel E.S., Mellon S.H., et al. Low serum IL-10 concentrations and loss of regulatory association between IL-6 and IL-10 in adults with major depression. *Journal of Psychiatric Research* 2009; **43**(11): p. 962-969.

131. Miller G.E., Rohleder N., Stetler C., and Kirschbaum C. Clinical depression and regulation of the inflammatory response during acute stress. *Psychosomatic Medicine* 2005; **67**(5): p. 679-687.

132. Weinstein A.A., Deuster P.A., Francis J.L., Bonsall R.W., et al. Neurohormonal and inflammatory hyper-responsiveness to acute mental stress in depression. *Biological Psychology* 2010; **84**(2): p. 228-234.

133. Benson S., Arck P.C., Blois S., Schedlowski M., et al. Subclinical depressive symptoms affect responses to acute psychosocial stress in healthy premenopausal women. *Stress-the International Journal on the Biology of Stress* 2011; **14**(1): p. 88-92.

134. Hennessy M.B., Deak T., and Schiml-Webb P.A. Early attachment-figure separation and increased risk for later depression: Potential mediation by proinflammatory processes. *Neuroscience and Biobehavioral Reviews* 2010; **34**(6): p. 782-790.

135. Garate I., Garcia-Bueno B., Madrigal J.L.M., Bravo L., et al. Origin and consequences of brain Toll-like receptor 4 pathway stimulation in an experimental model of depression. *Journal of Neuroinflammation* 2011; **8**.

136. Nguyen K.T., Deak T., Owens S.M., Kohno T., et al. Exposure to acute stress induces brain interleukin-1 beta protein in the rat. *Journal of Neuroscience* 1998; **18**(6): p. 2239-2246.

137. Ishikawa I., Kitamura H., Kimura K., and Saito M. Brain interleukin-1 is involved in blood interleukin-6 response to immobilization stress in rats. *Japanese Journal of Veterinary Research* 2001; **49**(1): p. 19-25.

138. Smith C., Wilson N.W., Louw A., and Myburgh K.H. Illuminating the interrelated immune and endocrine adaptations after multiple exposures to short immobilization stress by in vivo blocking of IL-6. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 2007; **292**(4): p. R1439-R1447.

139. Jankord R., Zhang R., Flak J.N., Solomon M.B., et al. Stress activation of IL-6 neurons in the hypothalamus. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 2010; **299**(1): p. R343-R351.

140. Marmot M.G., Smith G.D., Stansfeld S., Patel C., et al. Health inequalities among British civil servants - The Whitehall-II Study. *Lancet* 1991; **337**(8754): p. 1387-1393.

141. Marmot M. and Brunner E. Cohort Profile: the Whitehall II study. *International Journal of Epidemiology* 2005; **34**(2): p. 251-6.

142. Pan A., Sun Q., Okereke O.I., Rexrode K.M., et al. Depression and risk of stroke morbidity and mortality A meta-analysis and systematic review. *JAMA* 2011; **306**(11): p. 1241-1249.

143. Saleptsis V.G., Labropoulos N., Halaris A., Angelopoulos N.V., et al. Depression and atherosclerosis. *International Angiology* 2011; **30**(2): p. 97-104.

144. Low C.A., Thurston R.C., and Matthews K.A. Psychosocial factors in the development of heart disease in women: Current research and future directions. *Psychosomatic Medicine* 2010; **72**(9): p. 842-854.

145. Brim O., Baltes P., Bumpass L., and et a., *National survey of midlife development in the United States (MIDUS), 1995-1996 [Computer file]*, 1996, Harvard Medical School, Dept. of Health Care Policy.

146. Shaw B., Krause N., Chatters L., Connell C., et al. Emotional support from parents early in life, aging, and health. *Psychology and Aging* 2004; **19**: p. 4-12.

147. Stansfeld S., Head J., Bartley M., and Fonagy P. Social position, early deprivation and the development of attachment. *Social Psychiatry and Psychiatric Epidemiology* 2008; **43**(7): p. 516-526.

148. Cleary P.D., Goldberg I.D., Kessler L.G., and Nycz G.R. Screening for mental disorder among primary care patients - Usefulness of the General Health Questionnaire. *Archives of General Psychiatry* 1982; **39**(7): p. 837-840.

149. Koeter M.W.J. Validity of the GHQ and SCL anxiety and depression scales: A comparative study. *Journal of Affective Disorders* 1992; **24**(4): p. 271-279.

150. Stansfeld S.A. and Marmot M.G. Social-class and minor psychiatric disorder in British civil servants - A validated screening survey using the General Health Questionnaire. *Psychological Medicine* 1992; **22**(3): p. 739-749.

151. Cooper B. and Schwarz R. Psychiatric case-identification in an elderly urban population. *Social Psychiatry and Psychiatric Epidemiology* 1982; **17**(1): p. 43-52.

152. Hamer M. and Steptoe A. Association between physical fitness, parasympathetic control, and proinflammatory responses to mental stress. *Psychosomatic Medicine* 2007; **69**(7): p. 660-666.

153. O'Donnell K., Brydon L., Wright C.E., and Steptoe A. Self-esteem levels and cardiovascular and inflammatory responses to acute stress. *Brain Behavior and Immunity* 2008; **22**(8): p. 1241-1247.

154. Kirschbaum C. and Hellhammer D.H. Salivary cortisol in psychoneuroendocrine research - Recent developments and applications. *Psychoneuroendocrinology* 1994; **19**(4): p. 313-333.

155. von Kanel R., Kudielka B.M., Preckel D., Hanebuth D., et al. Delayed response and lack of habituation in plasma interleukin-6 to acute mental stress in men. *Brain Behavior and Immunity* 2006; **20**(1): p. 40-8.

156. Almela M., Hidalgo V., Villada C., van der Meij L., et al. Salivary alpha-amylase response to acute psychosocial stress: The impact of age. *Biological Psychology* 2011; **87**(3): p. 421-429.

157. Otte C., Hart S., Neylan T.C., Marmar C.R., et al. A meta-analysis of cortisol response to challenge in human aging: importance of gender. *Psychoneuroendocrinology* 2005; **30**(1): p. 80-91.

158. Vgontzas A.N., Bixler E.O., Lin H.M., Prolo P., et al. IL-6 and its circadian secretion in humans. *Neuroimmunomodulation* 2005; **12**(3): p. 131-140.

159.   Hamer M., O'Donnell K., Lahiri A., and Steptoe A. Salivary cortisol responses to mental stress are associated with coronary artery calcification in healthy men and women. *European Heart Journal* 2010; **31**(4): p. 424-429.

160.   Kidd T., Hamer M., and Steptoe A. Examining the association between adult attachment style and cortisol responses to acute stress. *Psychoneuroendocrinology* 2011; **36**(6): p. 771-779.

161.   Hubbard A.E., Ahern J., Fleischer N.L., Van der Laan M., et al. To GEE or not to GEE: comparing population average and mixed models for estimating the associations between neighborhood risk factors and health. *Epidemiology* 2010; **21**(4): p. 467-74.

162.   Zeger S.L. and Liang K.-Y. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* 1986; **42**(1): p. 121-130.

163.   Willett J.B., Singer J.D., and Martin N.C. The design and analysis of longitudinal studies of development and psychopathology in context: Statistical models and methodological recommendations. *Development and Psychopathology* 1998; **10**(2): p. 395-426.

164.   Adam E.K. and Gunnar M.R. Relationship functioning and home and work demands predict individual differences in diurnal cortisol patterns in women. *Psychoneuroendocrinology* 2001; **26**(2): p. 189-208.

165.   Taylor S.E., Karlamangla A.S., Friedman E.M., and Seeman T.E. Early environment affects neuroendocrine regulation in adulthood. *Social Cognitive and Affective Neuroscience* 2011; **6**(2): p. 244-251.

166.   Dickerson S.S. and Kemeny M.E. Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin* 2004; **130**(3): p. 355-391.

167.   Rabe-Hesketh S. and Skrondal A. *Multilevel and longitudinal modeling using Stata*. 2nd edition, 2008, College Station, TX: Stata Press.

168.   Llabre M.M., Spitzer S.B., Saab P.G., and Schneiderman N. Piecewise latent growth curve modeling of systolic blood pressure reactivity and recovery from the cold pressor test. *Psychophysiology* 2001; **38**(06): p. 951-960.

169.   Pruessner J.C., Kirschbaum C., Meinlschmid G., and Hellhammer D.H. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 2003; **28**(7): p. 916-931.

170.   Steptoe A., Willemsen G., Owen N., Flower L., et al. Acute mental stress elicits delayed increases in circulating inflammatory cytokine levels. *Clinical Science* 2001; **101**(2): p. 185-192.

171.   Van Ryzin M.J., Chatham M., Kryzer E., Kertes D.A., et al. Identifying atypical cortisol patterns in young children: The benefits of group-based trajectory modeling. *Psychoneuroendocrinology* 2009; **34**(1): p. 50-61.

172.   Kumari M., Badrick E., Sacker A., Kirschbaum C., et al. Identifying patterns in cortisol secretion in an older population. Findings from the Whitehall II study. *Psychoneuroendocrinology* 2010; **35**(7): p. 1091-1099.

173.   Nagin D.S. and Odgers C.L. Group-based trajectory modeling in clinical research. *Annual Review of Clinical Psychology* 2010; **6**(1): p. 109-138.

174.   Nagin D.S. *Group-based modeling of development*. 2005, Cambridge: Harvard University Press.

175.   Haviland A.M. and Nagin D.S. Causal inferences with group based trajectory models. *Psychometrika* 2005; **70**(3): p. 557-578.
176.   Nagin D.S. and Tremblay R.E. Analyzing developmental trajectories of distinct but related behaviors: A group-based method. *Psychological Methods* 2001; **6**(1): p. 18-34.
177.   Jones B.L., Nagin D.S., and Roeder K. A SAS procedure based on mixture models for estimating developmental trajectories. *Sociological Methods & Research* 2001; **29**(3): p. 374-393.
178.   Jones B.L. and Nagin D.S. Advances in group-based trajectory modeling and a SAS procedure for estimating them. *Sociological Methods & Research* 2007; **35**(4): p. 542-571.
179.   Radloff L.S. The CES-D Scale: A self-report depression scale for research in the general population. *Applied Psychological Measurement* 1977; **1**(3): p. 385-401.
180.   Heim C., Newport D.J., Wagner D., Wilcox M.M., et al. The role of early adverse experience and adulthood stress in the prediction of neuroendocrine stress reactivity in women: A multiple regression analysis. *Depression and Anxiety* 2002; **15**(3): p. 117-125.
181.   Stetler C. and Miller G.E. Depression and hypothalamic-pituitary-adrenal activation: A quantitative summary of four decades of research. *Psychosomatic Medicine* 2011; **73**(2): p. 114-126.
182.   Fries E., Hesse J., Hellhammer J., and Hellhammer D.H. A new view on hypocortisolism. *Psychoneuroendocrinology* 2005; **30**(10): p. 1010-1016.
183.   Uher R. The role of genetic variation in the causation of mental illness: an evolution-informed framework. *Molecular Psychiatry* 2009; **14**(12): p. 1072-1082.
184.   Zhang T.Y., Bagot R., Parent C., Nesbitt C., et al. Maternal programming of defensive responses through sustained effects on gene expression. *Biological Psychology* 2006; **73**(1): p. 72-89.
185.   Kudielka B.M. and Wust S. Human models in acute and chronic stress: Assessing determinants of individual hypothalamus-pituitary-adrenal axis activity and reactivity. *Stress-the International Journal on the Biology of Stress* 2010; **13**(1): p. 1-14.
186.   Dowlati Y., Herrmann N., Swardfager W., Liu H., et al. A meta-analysis of cytokines in major depression. *Biological Psychiatry* 2010; **67**(5): p. 446-457.
187.   Uher R., Caspi A., Houts R., Sugden K., et al. Serotonin transporter gene moderates childhood maltreatment's effects on persistent but not single-episode depression: Replications and implications for resolving inconsistent results. *Journal of Affective Disorders* 2011.
188.   Stevens H.E., Leckman J.F., Coplan J.D., and Suomi S.J. Risk and resilience: early manipulation of macaque social experience and persistent behavioral and neurophysiological outcomes. *Journal of the American Academy of Child and Adolescent Psychiatry* 2009; **48**(2): p. 114-27.
189.   McGowan P.O., Sasaki A., D'Alessio A.C., Dymov S., et al. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature Neuroscience* 2009; **12**(3): p. 342-348.
190.   Seckl J.R. and Holmes M.C. Mechanisms of Disease: glucocorticoids, their placental metabolism and fetal'programming' of adult pathophysiology. *Nature Clinical Practice Endocrinology & Metabolism* 2007; **3**(6): p. 479-488.

191.   Gotlib I.H., Joormann J., Minor K.L., and Hallmayer J. HPA axis reactivity: A mechanism underlying the associations among 5-HTTLPR, stress, and depression. *Biological Psychiatry* 2008; **63**(9): p. 847-851.

192.   Alexander N., Kuepper Y., Schmitz A., Osinsky R., et al. Gene-environment interactions predict cortisol responses after acute stress: Implications for the etiology of depression. *Psychoneuroendocrinology* 2009; **34**(9): p. 1294-1303.

193.   Way B.M. and Taylor S.E. The serotonin transporter promoter polymorphism is associated with cortisol response to psychosocial stress. *Biological Psychiatry* 2010; **67**(5): p. 487-492.

194.   Wells J.E. and Horwood L.J. How accurate is recall of key symptoms of depression? A comparison of recall and longitudinal reports. *Psychological Medicine* 2004; **34**(6): p. 1001-1011.

195.   Kirschbaum C., Pruessner J.C., Stone A.A., Federenko I.S., et al. Persistent high cortisol responses to repeated psychological stress in a sub-population of healthy men. *Psychosomatic Medicine* 1995; **57**: p. 468-474.

196.   van Eck M.M.M., Nicolson N.A., Berkhof H., and Sulon J. Individual differences in cortisol responses to a laboratory speech task and their relationship to responses to stressful daily events. *Biological Psychology* 1996; **43**(1): p. 69-84.

197.   Cohen S., Hamrick N., Rodriguez M.S., Feldman P.J., et al. The stability of and intercorrelations among cardiovascular, immune, endocrine, and psychological reactivity. *Annals of Behavioral Medicine* 2000; **22**(3): p. 171-179.

198.   Knudsen L.S., Christensen I.J., Lottenburger T., Svendsen M.N., et al. Pre-analytical and biological variability in circulating interleukin 6 in healthy subjects and patients with rheumatoid arthritis. *Biomarkers* 2008; **13**(1): p. 59-78.

199.   Musante L., Treiber F., Kapuku G., Moore D., et al. The effects of life events on cardiovascular reactivity to behavioral stressors as a function of socioeconomic status, ethnicity, and sex. *Psychosomatic Medicine* 2000; **62**(6): p. 760-767.

200.   Murali R. and Chen E. Exposure to violence and cardiovascular and neuroendocrine measures in adolescents. *Annals of Behavioral Medicine* 2005; **30**(2): p. 155-163.

201.   Bernstein D. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse & Neglect* 2003; **27**(2): p. 169-190.

202.   Burgermeister D. Childhood adversity: a review of measurement instruments. *Journal of Nursing Measurement* 2007; **15**(3): p. 163-76.

203.   Dellafemina D., Yeager C.A., and Lewis D.O. Child abuse - Adolescent records vs. adult recall. *Child Abuse & Neglect* 1990; **14**(2): p. 227-231.

204.   Hardt J. and Rutter M. Validity of adult retrospective reports of adverse childhood experiences: review of the evidence. *Journal of Child Psychology and Psychiatry* 2004; **45**(2): p. 260-273.

205.   Brown G.W., Craig T.K.J., Harris T.O., Handley R.V., et al. Validity of retrospective measures of early maltreatment and depressive episodes using the Childhood Experience of Care and Abuse (CECA) instrument - A life-course study of adult chronic depression - 2. *Journal of Affective Disorders* 2007; **103**(1-3): p. 217-224.

206.   Keyes K.M., Eaton N.R., Krueger R.F., McLaughlin K.A., et al. Childhood maltreatment and the structure of common psychiatric disorders. *British Journal of Psychiatry* 2012; **200**(2): p. 107-115.

207.   Catalano R.A., Saxton K., Bruckner T., Pearl M., et al. Hormonal evidence supports the theory of selection in utero. *American Journal of Human Biology* 2012; **In press**.
208.   Catalano R., Margerison-Zilko C., Goldman-Mellor S., Pearl M., et al. Natural selection in utero induced by mass layoffs: The hCG evidence. *Evolutionary Applications* 2012; **In press**.

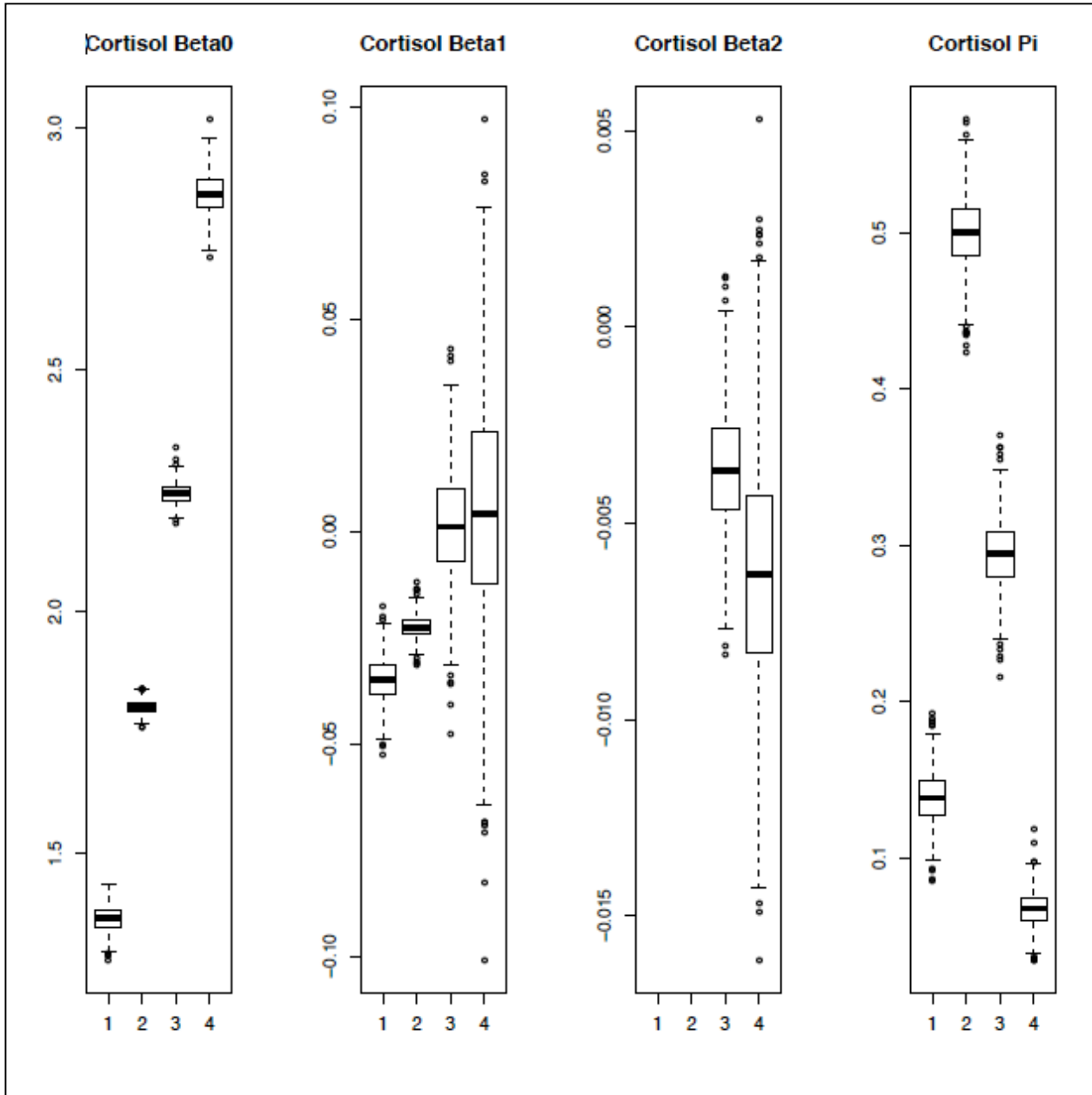**Appendix. Box plots of the bootstrapped cortisol and IL-6 model betas and group percentages (Pi ((π_j)).**

Figure 1. Bootstrapped parameter estimates (β_0, β_1, β_2, and π_j) for the fitted cortisol trajectory model. Numbers on the X axis refer to the trajectory group.
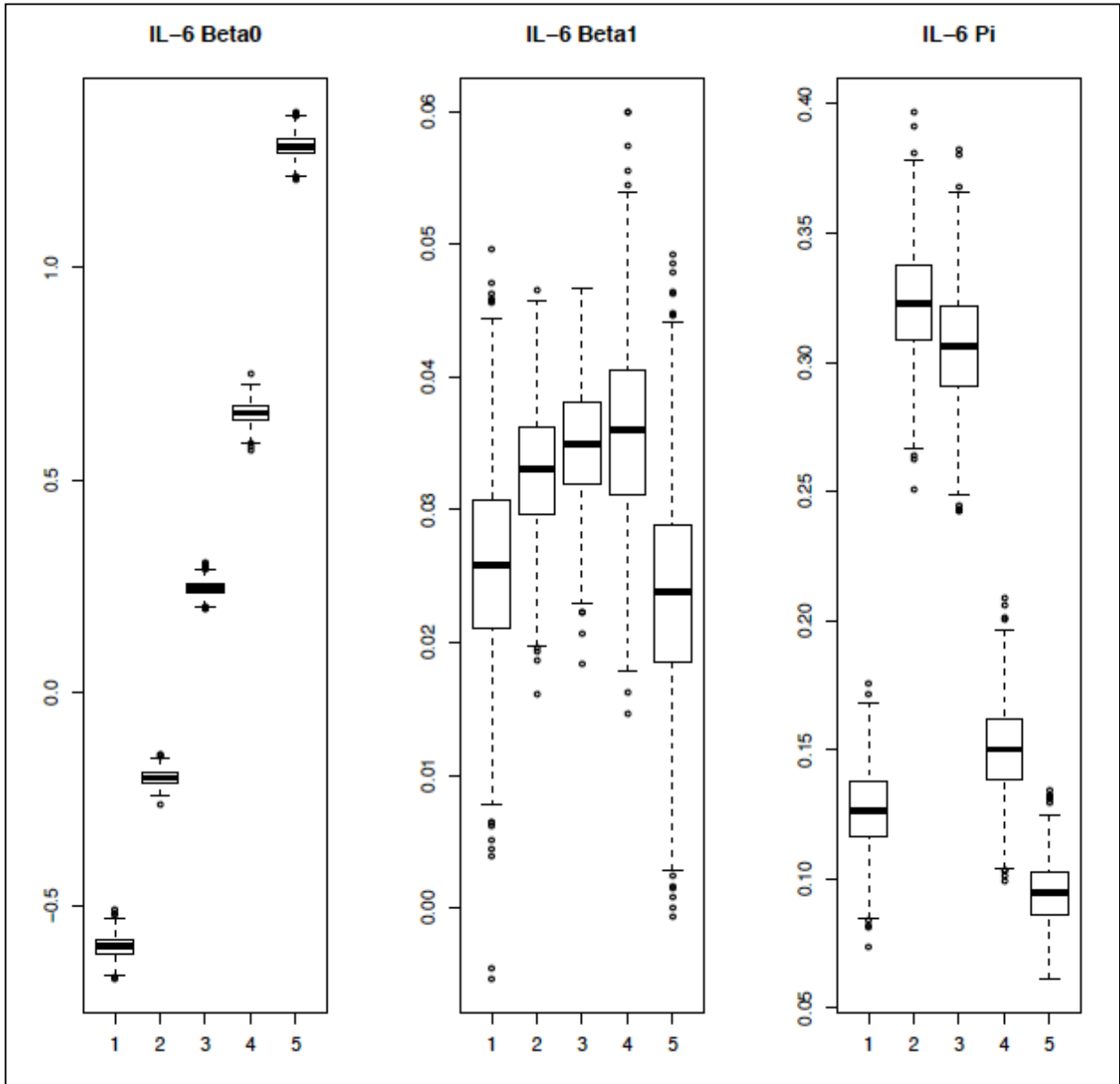
Figure 2. Bootstrapped parameter estimates (β_0, β_1, β_2, and π_j) for the fitted IL-6 trajectory model. Numbers on the X axis refer to the trajectory group.