Psychological and Neuroendocrine Determinants of Stress Regulation in Women

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The studies described in this thesis were performed at the department of Psychotherapy of the Riagg Rijnmond, Mental Health Clinic, Schiedam, The Netherlands and at the department of Psychiatry, Erasmus University Medical Center, Rotterdam, The Netherlands.

This research project was financially supported by the Stichting Psychoanalytische Fondsen, the Riagg Rijnmond and the Erasmus University Medical Center.

Layout: Optima Grafische Communicatie, Rotterdam, the Netherlands (www.ogc.nl)
Printed by: Optima Grafische Communicatie, Rotterdam, the Netherlands (www.ogc.nl)

ISBN: 978-90-8559-135-1

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Psychologische en neuro-endocriene factoren van stressregulatie bij vrouwen

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus

prof. dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaats vinden op

woensdag 15 maart 2017 om 15.30 uur

door

Jūratė Aleknavičiūtė
geboren te Šilutė
Litouwen

Erasmus University Rotterdam

PROMOTIECOMMISSIE:

Promotor:	Prof. dr. S.A. Kushner
Overige leden:	Prof. dr. H.W. Tiemeier
	Prof. dr. E.F.C. van Rossum
	Prof. dr. J.C.N. de Geus
Copromotoren:	Dr. J.H.M. Tulen
	Dr. C.G. Kooiman

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Chapter 1

General Introduction

INTRODUCTION

The worldwide lifetime incidence of mental illness is nearly 50% (1). Overall, the lifetime incidence of mental illness is similar in men and women (1). However, for a variety of mental health diagnoses, there are notable sex-associated distinctions. For example, mood disorders and psychological distress disproportionately affect women (2). Although it has been widely acknowledged that sex is an important marker of individual variability, sex-specific factors and the underlying biological mechanisms that impact resilience to stress and mental health, have not been sufficiently studied. Especially in women, this knowledge is very limited. Women, to a much greater extent than men, undergo hormonal fluctuations associated with the reproductive cycle. These fluctuations influence numerous bodily and mental functions, and have been suggested to be responsible for increased susceptibility to stress and stress-related disorders in women (1,2). This thesis focuses on hormonal factors involved in the physiological responses to acute psychosocial stress of women in the presence or absence of personality psychopathology, while also considering the influence of cognitive and genetic factors.

Psychosocial Stress

Many of the stressors we experience in our daily lives are psychological in nature and often socially oriented. Such stressors can include threat to social esteem, respect and self-worth, and/or acceptance within a group, or a threat that we feel we have no control over. Psychosocial stress has been defined as a real or interpreted socially-oriented conditioned threat to the psychological integrity of an individual, which induces biochemical, physiological, cognitive and behavioral changes (3). This response to stress represents an integrated reaction to stressors and is essential to adapt to various homeostatic challenges. Effective adaptation to stress requires a complex interplay of several factors, which include a dynamic interaction between environmental demands, the individual's capacity to cope with those demands, and the individual's appraisal of that relationship (3,4). Subsequently, cognitive appraisal is considered a central concept in explaining psychological stress (4).

It is widely accepted that individuals vary significantly in the way they react to a demanding natural environment, or complex social interactions. It is also acknowledged that these individual differences might be associated with behavior and health outcomes (5,6). Given the significant variability in the strength and valence of emotional reactions and biological system activity, the identification and mechanistic understanding of individual differences has become an important challenge for psychiatric research (7). The capacity to properly contextualize and monitor a

situation has been shown to be essential to ascertain whether specific situations are threatening for an individual's well-being.

Cognitive stress appraisal

Already decades ago, the transactional stress model postulated that cognitive appraisal processes are key concepts in determining appropriate coping mechanisms and enable adaptation to the environment (2). Which coping mechanism is selected to be employed is determined by how an individual appraises a stressful event, and his or her adaptive reaction to stress. In other words, appraisal mediates the stressfulness of events. The cognitive appraisal of stressors is a process of evaluation comprising two stages. Primary appraisal is concerned with the subjective assessment of the demands of the environment, for example, whether there is potential for harm or benefit. Secondary appraisal involves an individual's determination of his or her resources that can be applied to the situation as coping options. These stages interact to produce an overall perception, management, and optimally, termination of stress (8). Thus, a stress reaction takes place when the individual concludes that environmental stimuli are exceeding his or her personal coping capacities.

These cognitive stress appraisals are an effortless and automatic interpretation of the perceived situation that creates an emotional experience and allows the individual to respond adaptively. Hence, the motivation to adapt to environmental demands involves complex and dynamic interaction networks among emotions, cognitive appraisals, physiological responses and behavioral experiences (4,9). Additionally, events appraised as highly significant are more likely to result in psychophysiological stress reactions (10–12). From a developmental perspective, temperament and attachment are thought to be major organizers of early social-emotional development and are important factors in an individual's psychosocial functioning. It has been suggested that both attachment and temperamental factors can make unique and interactive contributions to how an individual deals with a demanding environment (13,14). However, knowledge of the role of attachment and temperament on the cognitive processing of psychosocial stress in women, and in particular with regarding to the influence of personality psychopathology, has remained limited.

Personality Psychopathology

Personality disorders are 'pervasive, inflexible, maladaptive' collections of traits that impact an individual across a broad range of situations (15). Personality disorders exhibit high comorbidity with Axis I pathology (16,17). Personality disorders are heterogeneous regarding their clinical features and etiology. The symptoms of personality disorders are caused by multiple factors such as inborn temperamental traits, as well as environmental and developmental events (18). Therefore, the common

traits of chronic, inflexible styles of perceiving oneself and interacting with others vary widely in presentation. There are ten categories of personality disorders defined within the DSM 5 (19,20). Of those, Borderline Personality Disorder (BPD) and Cluster C (avoidant/dependent) Personality Disorder (CPD) are among the most common in clinical samples (21,22).

Borderline Personality Disorder (BPD)

BPD is considered to be the most complex, and certainly one of the most devastating, personality disorder categories (23,24). It is also by far the most intensively studied. Approximately 2-4% of the general population suffers from BPD (25). However, BPD is more common in Axis I clinical populations, with estimated prevalence rates of 9 to 23% in psychiatric outpatients (25) and up to 44% in psychiatric inpatients (26). Female patients predominate within psychiatric settings (about 75%), however men are more commonly diagnosed with BPD in substance abuse or forensic settings (27,28).

Patients with Borderline personality disorder (BPD) have long been recognized as creating considerable challenges for clinicians who diagnose and treat them (29). The main reasons for the treatment difficulties encountered are patterns of intense affectivity, destructive relationships, impulsive behavior, and problems with mentalization that make it difficult for patients to reflect upon these patterns (30). There is growing evidence that emotional dysregulation is a core feature in BPD (31). Ever-changing emotions, together with poor social cognition, contributes to an unstable sense of the self and unsteady social interactions (32). In turn, psychosocial deficits reinforce emotional dysregulation, in this way creating a circular mechanism.

Cluster C Personality Disorder (CPD)

All patients with CPD exhibit anxiety in some form (33). Cluster C personality disorders, including avoidant, dependent, and obsessive-compulsive personality disorders, are reported to be among the most common mental disorders in the general population (34). Whether caused by fear of judgement by others, or abandonment, patients with CPD suffer from uncomfortable beliefs and sensations that cause distress and interfere with their functioning (35). Patients with CPD usually have a less problematic course in therapy than patients with BPD and are considered clinically less disruptive (33). However, they also often remain in a passive patient role, without making the necessary efforts to succeed in treatment (35). Furthermore, cluster C disorders (dependent and avoidant) generally have been regarded as disorders of medium severity (33). However, this assumption has not been thoroughly studied in empirical studies.

Dysfunctional affect regulation as a common feature of BPD and CPD

Despite the amount of research on the benefits of successfully regulating affect for our mental and somatic well-being (36), research on the effects of dysfunctional affect regulation in psychiatric patients remains inconclusive. Yet, it has been established that affect dysregulation is involved in the etiology and maintenance of psychopathology (37). In addition, dysfunctional affect regulation is often described in patients with complex psychopathology, such as the presence of a combination of DSM Axis I and Axis II symptoms. People with BPD and/ or CPD are not capable of establishing and maintaining interpersonal relationships, which require sufficient affect regulation (38).

Whereas BPD is a classic example of a global dysregulation of negative affect, primarily involving fear and anger, patients with CPD exhibit avoidant behavior and unmodulated affect accompanied by severe anxiety, shame and panic (39,40). Individuals with BPD and CPD are considered to have a reduced capacity to relax after stressful situations, which subsequently reinforces their hyper-aroused state in a dysregulated manner (41,42). As a consequence, the perception of threat is usually elevated and the symptoms of BPD and CPD is typically exacerbated by stress. The limited ability to process information consequently contributes to poor self-perception and coping, and reduces control over affect and impulses.

Although there is ample evidence that patients with BPD and CPD experience emotional dysregulation, the evidence for biological sensitivity is more ambiguous (43). Data about the concomitant circumstances and mechanisms that underlie emotional dysregulation is sparse and inconclusive. Distinct studies using psychobiological markers of emotion have thus far failed to identify a consistent physiological pattern of affect dysregulation in BPD versus CPD (44,45).

Affect dysregulation and developmental components

Although multiple interdependent processes are involved in the regulation of emotions, dysfunctional affect regulation has been hypothesized to result from childhood adversity and the quality of early-life attachment, most notably neglect or abuse by primary caregivers (46–48). Such adverse events during early-life development have been suggested to result in an insecure attachment style. A healthy attachment bond has been suggested to be of vital importance for developing adaptive emotional control (46,47). Whereas a secure attachment style is theorized to be related to a more adaptive regulation of affect, an insecure attachment style is thought to impair the development of affect regulation, cognition and coping in emotional relationships (24,47,49).

The biological stress systems

The ability to respond to the demands of a situation with a general alarm response is one of the essential elements in the global adaptive and self-regulating systems of biological organisms (50). Over the course of evolution, overlapping mechanisms have developed, to deal with environmental demands. In mammals, the autonomic nervous system (ANS) and the Hypothalamic-pituitary-adrenal (HPA) axis are considered to be the most important systems, but many other endogenous stress-reactive systems contribute. There are also several other systemic processes, such as prolactin release (51) and/or circulating IL-6 levels (52) that have temporal links to stressful stimuli. Another important system is the Hypothalamic-Pituitary-gonadal (HPG), which regulates the release of gonadal steroid hormones (53). This thesis work focuses on the two major stress regulating systems (ANS and HPA axis), and the functionally interconnected reproductive system (HPG axis) (Figure 1).

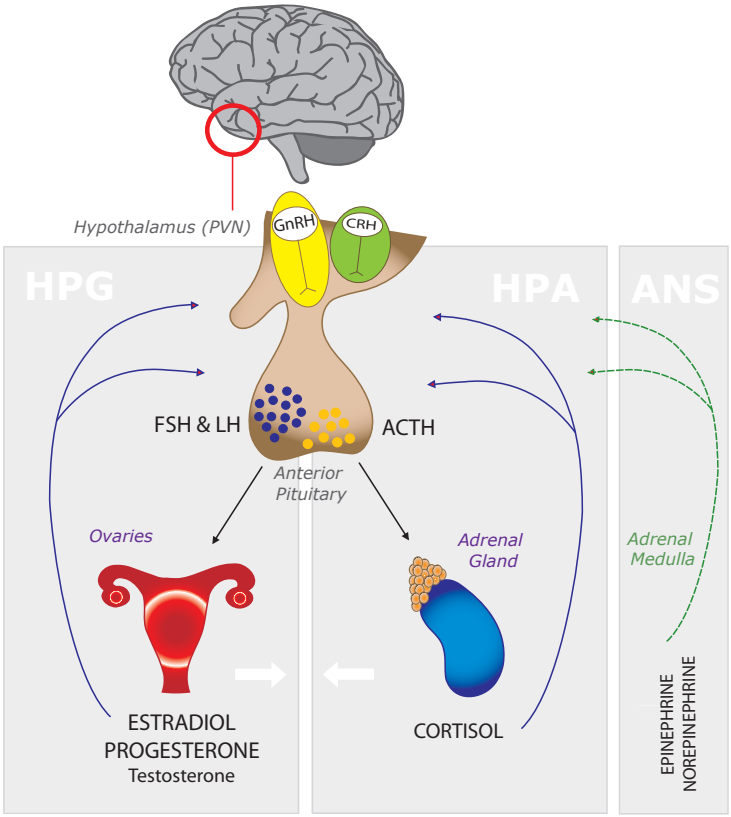

Figure 1. A schematic diagram of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes. The autonomic nervous system (ANS) is also an important modulating factor. The HPA and HPG axes share a common bipartite composition, with both central (hypothalamic stimulation of the pituitary in both) and peripheral (gonads and adrenals, respectively) components.

The Hypothalamic-Pituitary-Adrenal axis

The HPA axis is the primary neuroendocrine system governing how mammals cope with and adapt to stressors. Activation of the HPA axis represents a primary hormonal response to a homeostatic challenge. Thus, the exposure to a stressful situation results in a wide spectrum of central and peripheral responses starting with corticotrophin releasing hormone (CRH), which is secreted by the paraventricular nucleus of the hypothalamus. Released CRH triggers the pituitary gland to secrete the adrenocorticotropic hormone (ACTH). In turn, ACTH triggers the adrenal cortex to produce the glucocorticoid cortisol. Released cortisol, via negative feedback, suppresses CRH and ACTH secretion from the hypothalamus and the pituitary gland (53). Cortisol is the final output of the HPA axis and therefore an important dynamic index of the state of the HPA axis.

Cortisol acts principally in two different ways: basally through support of normal metabolic and diurnal functions, and dynamically in response to stress (53,54). The basal function is driven by hypothalamic input and is very sensitive to negative feedback control. The stress regulatory function of cortisol secretion is influenced by the amygdala and hypothalamus, but is notably less sensitive to negative feedback (52,53).

Although the majority of cortisol, up to 90 percent, is bound to the proteins corticosteroid-binding globulin and albumin, the remaining unbound cortisol constitutes the biologically active fraction (55,56). Saliva sampling is a reliable indicator of free unbound cortisol concentration in the blood (55), as only free unbound cortisol passes into the saliva. Analogously, only free unbound cortisol is capable of passing through the blood-brain barrier to mediate effects within the central nervous system (54). Although cortisol has many well-demonstrated benefits during acute periods of threat and stress, chronically elevated cortisol levels have considerably deleterious systemic consequences (57). Therefore, a tightly-regulated stress response is very important, as inappropriate or prolonged HPA axis activation has been associated with numerous pathophysiological and psychopathological disease states (58,59).

The Autonomic Nervous System

The ANS functions importantly in regulating physiological arousal and inhibition during stress. The ANS consists of two main branches: the sympathetic and parasympathetic nervous system. When exposed to a stressor, the sympathetic branch is rapidly activated and endows an individual with a readiness to respond. This rapid response is mostly involved in regulating arousal by release of adrenaline and noradrenaline, which in turn stimulate heart rate and blood pressure, dilate the pupils, and activate the sweat glands. This branch is often referred to as the “fight-or-flight’ system. Although adrenaline and noradrenaline are unable to cross blood-brain barrier, they directly

stimulate the vagal nerve, a primary component of the parasympathetic system that innervates the sinoatrial node of the heart. The parasympathetic branch of the ANS is responsible for conservation of energy and regulating organ functions when the body is at rest. Both branches are constantly active, operate independently of one another, and exert reciprocal influences on the heart (60). The magnitude of activity varies depending upon internal and environmental conditions. When active coping of the individual is required, the sympathetic system inhibits vagal tone to support an increase in heart rate. Afterwards, vagal tone is restored, thereby regulating heart rate back to resting levels.

These responses are meant to help the body to adapt to and protect against stressful stimuli (61), but chronic excessive (sympathetic) activation can produce neurochemical imbalances that may contribute to the development of psychiatric disorders (62). These physiological responses have been suggested to be an important physiological marker of psychological states, such as the subjective feeling of anxiety or emotional dysregulation (63). Heart rate and skin conductance level (SCL) are reliable typical indices of ANS activity. Whereas SCL is an established biomarker of sympathetic nervous system activity, mean heart rate reflects innervation of both sympathetic and parasympathetic systems.

The Hypothalamic-Pituitary-Gonadal axis

The HPG axis is a neuroendocrine axis that functions parallel to the HPA axis and regulates reproduction. The reproductive and stress systems have an analogous bipartite composition, with both central (hypothalamic stimulation of the pituitary in both) and peripheral (gonad and adrenal glands, respectively) components (53). The hypothalamus directs many of its actions through the pulsatile secretion of gonadotropin-releasing hormone (GnRH), which in turn acts on the pituitary gland to stimulate the synthesis and release of gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (64,65).

In men, gonadotropins circulate systemically and constitutively. Circulating gonadotropins act on the testes to release testosterone, which in turn, together with gonadotropins, negatively regulates hypothalamic function to maintain homeostasis. The female reproductive system is undoubtedly more complex. The hypothalamus releases GnRH, and secreted gonadotropins trigger the ovaries to release estradiol and progesterone (64,66). To initiate the ovulatory cascade, women experience hormonal surges, a uniquely sex-specific physiological phenomenon, in which estrogen switches from exerting negative feedback to positive feedback (64). Following ovulation, progesterone levels rise higher than estradiol, and then both estrogen and progesterone levels slowly decrease together. The declining levels of estrogen then reverse the negative feedback on GnRH, initiating the cycle to begin anew (64).

HPA and HPG axis interactions during stress

Studies on interactions between the stress and reproductive axes have primarily focused on the mechanisms by which stress impacts the reproductive system. Stress, whether psychological or physical, has been suggested to disturb the reproductive axis at every level of the axis, from the hypothalamus to the ovaries or testes (64,67). However, the relationship between these two endocrine systems is not unidirectional. Recently, a reciprocal relationship between the HPA and HPG axes has been shown, suggesting a functionally interconnected reciprocal co-regulation between them (68). Consequently, changes in sex steroid levels modulate the magnitude of the stress response.

A consistent finding is that men exhibit two-fold higher cortisol responses to psychosocial stress compared to women (69), when menstrual cycle phase or oral contraceptive use is not included as a confounding factor. Therefore, it has been suggested that the internal sex-specific endocrine milieu is related to variation in responses to stress. Endogenous levels of sex steroids and exogenous administration of sex hormones have been shown to affect HPA axis responses (70). Several studies have shown that the salivary cortisol response to psychosocial stress in women is modulated by the phase of the menstrual cycle. Women in the luteal phase have salivary cortisol stress responses comparable to those of men, whereas women in the follicular phase exhibit significantly lower salivary cortisol responses, comparable to those of women using oral contraceptives (71–75). Furthermore, although estradiol seems to have the most potent effects on HPA axis-mediated stress regulation, progesterone was also observed to be an important regulator of HPA axis function by enhancing stimulated HPA axis function in women (76,77). Remarkably, however, despite the potency by which sex steroids regulate glucocorticoid release, knowledge of how sex steroids operate to regulate the HPA axis is not well established. The effects of HPA axis activation on sex steroid levels also remain to be investigated.

Determinants of psychophysiological stress reactivity

Genetic factors

It is assumed that genetic factors are among the most important factors in determining an individual's adaptive response to stress. Various studies imply that the serotonin (5-hydroxytryptamine; 5-HT) neurotransmission system and the HPA axis are closely interrelated, and that both function importantly in the mediating responses to stress (78,79). The serotonin transporter (5-HTT) regulates the concentration of 5-HT in the synaptic cleft and has been shown to contribute to many physiological functions (80). 5-HTT is encoded by a single gene (*SLC6A4*), within which the 5-HTT-linked polymorphic region (5-HTTLPR) regulates the *SLC6A4* transcriptional activity (80). There are two major variants of the 5-HTTLPR, which differ significantly in their

functional efficiency. The long (L) allele of the 5-HTTLPR is related to higher transcriptional efficiency and higher 5-HTT expression, compared to the short (S) allele (80). Previous studies have suggested that 5-HTTLPR variations moderate the stress response, with dominance of the S allele over the L allele (81). However, there are conflicting results (82). Therefore, further clarification of the impact of 5-HTTLPR variant on stress reactivity is needed.

Gene and environment interaction

Following the diathesis-stress theory, some studies have suggested that HPA axis and stress reactivity might be modulated by the interaction of genetic vulnerability and major life stressors (81,83,84). The majority of these studies imply that 5-HTTLPR genotype modulates HPA axis reactivity to social stress. However, the direction of this interaction remains inconclusive. Many factors such as gender, age, and cumulative exposure to stressful life events, have been suggested to contribute to the magnitude and direction of *SLC6A4* gene 5-HTTLPR x environment interactions on HPA axis responsivity (82).

In addition, research has shown that childhood trauma exposure such as physical and sexual abuse, emotional neglect, and early relationship losses can have detrimental effects on the developing brain. Specifically in early childhood, the developing HPA axis is under strong social regulation and vulnerable to environmental disturbances (85). Various studies have postulated that early life adversities have long-lasting effects on the activity of the HPA axis (86–88). Furthermore, childhood trauma is considered an important precursor to many forms of pathology in adulthood (89) and is prospectively related to a range of personality psychopathology symptoms and diagnoses (90).

Hormonal contraceptives

Currently, psychoneuroendocrine research takes into account the potential impact of the menstrual cycle on salivary cortisol responses to stress (74). However, adjusting for the effects of hormonal contraceptives on cortisol responses remains difficult, is sometimes overlooked, and often simply established as an exclusion criterion.

Worldwide, more than 70 million women of reproductive age are estimated to use some form of hormonal contraception. Remarkably however, the effects of synthetic steroids – the active component of hormonal contraceptives – on the physiological response to stress have scarcely been investigated. These studies have demonstrated that women using oral hormonal contraceptives (typically containing estrogen and progestin) displayed a blunted salivary cortisol response following acute stress (73,92,93), or following pharmacological stimulation (75), compared to women in the luteal phase of menstrual cycle.

More recently, women have been increasingly expressing preference for long-lasting reversible contraceptives such as progestin releasing intrauterine devices

(IUD) (94), making them the fastest-growing method of hormonal contraception. However despite their widespread and increasingly frequent use, data on the impact of progestin-only contraception on the functioning of the HPA axis in women is almost entirely unknown. Obviously, given our increasing understanding of the physiologically important co-regulation of the HPA and HPG axes, knowledge of the corresponding influence of hormonal contraceptives on female physiology is of compelling importance, to women themselves, to their clinicians, as well as for clinical and fundamental research.

Rationale and aims of the thesis

Women are particularly susceptible to stress-related disorders, and the impact of hormones, natural or synthetic, could be a crucial factor to include when studying women's mental health. Yet, both natural hormonal fluctuations and the use of hormonal contraceptives have long been considered valid scientific arguments to exclude women from studies regarding stress physiology. Therefore, we explicitly focused our studies of stress regulation to include women, by directly considering the influence of the menstrual cycle and contraceptive use. Furthermore, given that maladaptive emotional control is a significant burden to women affected by personality disorders, this thesis also aimed to investigate stress regulation in women with personality psychopathology by examining psychophysiological responses to acute psychosocial stress in relation to its cognitive and genetic determinants. In a cohort of women recruited among outpatients with personality disorder and matched healthy controls, we assessed cognitive appraisal, genetic factors, subjective mood, cortisol, and autonomic nervous system responses during a standardized psychosocial stress procedure. In addition, we aimed to explore biological determinants of physiological stress reactivity in women by performing an ACTH challenge test in healthy women.

Aims of the thesis

The specific aims of this thesis are as follows:

- Given the potential of cognitive appraisal to either facilitate or impede stress coping capacity, we aimed to consider fundamental personality characteristics that could be as potential determinants of cognitive appraisals to acute psychosocial stress in women with regard to their personality disorder burden.
- Since 5-HT is considered an important neurotransmitter regulating the HPA axis response to stress and has been implicated in various stress related disorders, we sought to examine the effects of the *SLC6A4* 5-HTTLPR genotype on salivary cortisol responses to psychosocial stress in women with personality disorder and healthy controls.

- Salivary cortisol levels, mean heart rate, SCL and subjective mood were studied before, during and after acute psychosocial stress to clarify potential differences in stress regulatory systems between distinct clusters of personality disorder (cluster C and cluster B) and healthy controls. In addition, considering the high rates of early life adversities in the patient samples, we also explored the impact of these adversities on the physiological responses to acute psychosocial stress.
- In developed countries, about 50% of all women of reproductive age rely on some method of hormonal contraception. We aimed to investigate the impact of these exogenous hormones on the physiological responses to psychosocial stress, by studying the functioning of the HPA axis at central and peripheral levels. In addition, we examined long-term stress exposure under naturalistic conditions using hair cortisol measurements. We studied healthy women in two distinct hormonal contraceptive groups (oral monophasic combined preparations containing ethinylestradiol and levonorgestrel, and the levonorgestrel-releasing IUD) as well as in naturally cycling women.
- Lastly, we were interested in advancing our understating of how HPA axis activation might influence HPG axis activity. By administering a low-dose of ACTH to healthy women using different contraceptives, we aimed to further dissect the hormonal context by which the adrenal cortex activity mediates gonadotropin release. Serum steroid and gonadotropin concentrations were measured prior to, and after, intravenous ACTH administration.

Outline of the thesis

In *Chapter 2,* the associations between fundamental personality characteristics (attachment styles, temperament) and cognitive appraisals of acute psychosocial stress in women with and without personality disorder were explored. In order to understand the individual differences in cognitive appraisal of acute psychosocial stress, we constructed a model linking personality characteristics to cognitive appraisals while controlling for maladaptive personality traits. In *Chapter 3,* the impact of the genetic factor *SLC6A4* 5-HTTLPR on the endocrine stress response in women with and without personality disorder was investigated. The study described in *Chapter 4* examines psychophysiological responses to acute psychosocial stress in two different clusters of personality disorder, cluster B and cluster C, in comparison to healthy controls. *Chapter 5* investigates the systemic physiological influence of hormonal contraception in healthy women, with emphasis on the levonorgestrel-releasing IUD. The study in *Chapter 6* investigates the effects of low-dose ACTH test on gonadotropin release. Finally, the main findings and conclusions of the studies are presented and discussed in *Chapter 7* in which the research implications and suggestions for future research are addressed.

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Chapter 2

Maladaptive personality traits mediate cognitive appraisal during stress

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ABSTRACT

Attachment and temperamental factors are considered to contribute to how an individual negotiates demanding environments. However, the influence of attachment and temperament on the cognitive processing of psychosocial stress in women remains incompletely understood. Using structural equation modeling we examined the direct and indirect impact of attachment insecurity and temperament on the cognitive appraisals of acute psychosocial stress in women with high and low burdens of psychopathology. Additionally, the mediating role of maladaptive personality traits was considered. Female outpatients with a personality pathology (N= 102) and healthy women (N= 96) were recruited. Cognitive appraisal was assessed during exposure to acute psychosocial stress in a laboratory setting. Our findings revealed that positive affectivity was directly linked to secondary appraisal of acute psychosocial stress. Maladaptive personality traits mediated the negative impact of both attachment anxiety and negative affectivity on primary appraisal of acute psychosocial stress. Notably, this pattern of associations was independently valid in both the patient and control samples. These findings confirm that positive affectivity buffers acute psychosocial stress. Furthermore, the results suggest that maladaptive personality traits are important factors in understanding the relationships between attachment, temperament, and mentalization capacity in stressful contexts, not only in clinical samples, but also in the general population.

INTRODUCTION

Adaptation to our environment involves complex dynamic interaction networks comprising emotions, cognitive processes, behavioral experiences and physiological responses (1,2). Research examining the utility of cognitive models underlying psychological adjustment to environmental demands has shown that cognitive factors can interact significantly with stressors in the prediction of psychological adaptation (3-5). In addition, more recent studies have suggested a proximal impact of cognitive appraisal processes in mediating physiological responses following a stressor (6-10). These findings suggest that cognitive processes can be seen as important elements to influence the risk of, and resilience against, maladaptive health outcomes.

Cognitive appraisal during stress

The transactional stress model of Lazarus and Folkman (1984) remains a leading model to explain how environmental conditions influence adaptive functioning and well-being. This model posits that an individual, when opposed with a threatening or challenging situation, undergoes specific cognitive processes: appraisal of threat or challenge (how dangerous is the situation) and evaluation of coping resources (what are my capabilities to handle a demanding situation adequately). These cognitive appraisal processes are an effortless and automatic interpretation of the perceived situation and are considered to occur as a result of the interaction between situational and personality characteristics (11). Interestingly, few research efforts have been made to pinpoint the dispositional personality characteristics contributing to this interaction.

Attachment and Temperament: critical factors for psychosocial functioning

According to the stress-diathesis theory, individual differences in reactivity to stressful events are dependent on personality characteristics which might buffer, or on the contrary exacerbate, emotional upheaval and ultimately the development of psychiatric decompensation (12-14). Among the major factors leading to individual differences stress reactivity are temperamental biases which appear to be innate and stable over time (15-17); DSM-5 section III alternative model for personality disorders). Another personality-related aspect of clinical relevance is the concept of attachment style. In mammals, the tendency to develop an affective bond with a primary caretaker who protects and soothes an infant in distress, is considered to be inborn (18). Depending on experiences and interferences in (early) development, each infant/individual develops so-called working models or basic assumptions of oneself and others which establish his/her appraisal process, and psychological and physiological reactions to stress (19,20). Secure working models are theorized to be related to more flexible appraisal, appropriate emotional arousal and realistic interpretations of experiences,

together leading to constructive coping strategies. Conversely, insecure working models are associated with more rigid and rapidly established negative appraisals and inappropriate emotional arousal (21,22). In the psychotherapeutic literature, these working models are considered the building blocks of character and its related pathology, with insecure attachment being related with inadequate capacities to mentalize that in turn hamper adequate stress regulation.

Whereas attachment and temperament are considered to be related constructs and suggested to contribute to individual differences in organizing and regulating thoughts, perceptions and emotions (23-26), very few studies have investigated the associations between personality characteristics and cognitive processes when an individual is exposed to acute psychosocial stress. Whereas emotional arousal or psychosocial stress has been suggested to impair mentalizing ability (27,28), the influence of basic personality characteristics such as attachment style or temperament on cognitive resources remains insufficiently determined.

Some studies of attachment and cognitive appraisal have demonstrated that threat appraisal differs between individuals as a function of attachment style during both attachment- and non-attachment-related stressors (29-31). Anxiously attached individuals exhibit hyper-reactivity to stress, tend to exaggerate their helplessness and vulnerability, and also are much more likely to ruminate over the stressful event (20). In contrast, individuals high in attachment avoidance are associated with emotional inhibition or suppression, the dismissal of threatening events, and a tendency to trivialize distress (20). Correspondingly, temperament might be considered to shape an individual's appraisal of stress (32-34), and to predict or mediate distress (35-37). However, studies on the association between temperament and cognitive appraisal of acute stress are also scarce. Nevertheless, there is evidence that temperamental traits are related to an individual's attitude and approach to life. Several reports demonstrate that positive affectivity, as reflected in an optimistic and energetic approach to life, is associated with positive stress appraisal and flexible adaptation to changing environmental demands (38-42).

Impact of maladaptive personality traits

Alternatively, individuals who express high negative affectivity tend to evaluate situations as threatening, uncontrollable or overall in negative terms (43). In addition, negative affectivity has been shown to play an important role in many forms of Axis I and Axis II psychopathology (44,45). Negative affectivity has been suggested to be related to the affective instability concept, which involves extreme shifts in mood, and disturbances in affect intensity and stability (46). Affective instability is an important feature in several forms of psychopathology and is widely described in the psychiatric literature (47,48). Affective instability might be considered a trait-like dimension or

a symptom profile representing a change from premorbid state. Notably, it has been suggested that affective instability uniquely predicts individual functioning even after controlling for the temperamental factor (49). To that end, dysfunctional personality traits such as emotional dysregulation might be more predictive than temperament traits in the responding to and approaching of stressful events. Although maladaptive personality traits are often found to be prominent in psychopathologies, they are not specific for clinical populations. Subclinical levels of dysfunctional traits are found in a substantial percentage of the general population (50).

Present study

Taken together, both attachment and temperament are thought to be major organizers of early cognitive-emotional development and are important factors in how an individual deals with a demanding environment. Thus far, however, little attention has been given to the personality aspects involved in stress induced cognitive processes. Therefore, the aim of the present study was to explore the role of attachment and temperament on cognitive appraisals of acute psychosocial stress by developing an integrative model linking attachment insecurities (i.e., attachment related anxiety and avoidance) and temperament (positive and negative affectivity) to cognitive appraisals of acute psychosocial stress. In addition, we included in our model maladaptive personality traits (emotional dysregulation and dissocial behaviour) as a mediating variable in order to better understand the impact of dysfunctional personality traits on cognitive appraisals of an acute stressful situation. In order to explore the clinical relevance of our model, we included two female samples: healthy females and females with a personality psychopathology. There is considerable evidence that cognitive appraisal and coping capacity with stress can be influenced by childhood trauma (51) as well as by temporary symptoms of anxiety and/or depression (52). Therefore, we performed sensitivity analyses to exclude the potential effects of psychological distress and childhood trauma on the model.

Psychosocial stress

To induce acute psychosocial stress, we used a well-established paradigm, the Trier Social Stress Test (TSST)(53). The TSST employs a combination of two important elements, i.e. social-evaluative threat and uncontrollability (54). The TSST has been shown to be a reliable test to induce moderate psychosocial stress in a laboratory setting by challenging the participant's self-esteem in interpersonal situation. Additionally, the TSST has been shown to address an important aspect of individual's self-identity including valued traits and abilities (53,54). A number of theories support the notion that humans are driven to preserve the social self and are vigilant to threats that may endanger their social esteem and status (18,54,55).

MATERIALS AND PROCEDURES

Participants

The study sample comprised 198 female participants aged 18-45 years. Healthy female controls (n=96) were recruited through posted flyers and local internet advertisements. Women with a personality psychopathology (n=102) were recruited from the outpatient clinics for mental health in Rotterdam. Diagnoses were made by experienced psychotherapists, based on the Axis II DSM-IV criteria (56). Patients were considered ineligible to participate if they had a medical or comorbid diagnosis of bipolar disorder, schizophrenia or current major depression. Healthy female controls had no DSM-IV Axis I or Axis II diagnoses and without any history of psychiatric or psychological treatment. All participants were native Dutch speakers, of which the majority were Caucasian (n=187, 94.4%).

Written informed consent was obtained from all participants. The study was conducted according to the declaration of Helsinki and was approved by the Medical Ethical Research Committee of the Erasmus MC, University Medical Center Rotterdam. Participants were evaluated in two structured visits. During the initial visit, all subjects provided sociodemographic data and completed questionnaires regarding their general medical health, severity of personality pathology, attachment style, and temperament. During the second visit, the Primary Appraisal Secondary Appraisal scale (PASA) was administered immediately prior to the TSST.

Questionnaires

Attachment

The revised version of the Experiences in Close Relationships (ECR-r) is a self-report questionnaire with 36 items for the assessment of attachment-related anxiety and avoidance (57,58). Higher mean scores indicate greater degrees of attachment related anxiety and/or avoidance, indicating attachment insecurity. Low scores on both dimensions are considered to indicate attachment security. Participants were asked to think about their romantic partner while rating the appropriateness of each item on a 7-point Likert scale. Participants without a current partner were asked to rate how they felt generally during intimate relationships. The ECR-r is a frequently used self-report questionnaire to assess attachment style and is considered to have good psychometric properties (57-60).

Temperament

The Positive and Negative Affect Scale (PANAS) (43,61) reflects affective processes, consistent with most conceptualizations and operational definitions of temperament (62). The PANAS comprises 20 items, with 10 items measuring positive affectivity (PA:

e.g. energetic, inspired) and 10 items measuring negative affectivity (NA: e.g. angry, upset). Each item is rated on a 5-point Likert Scale, ranging from 1 (*Not at all*) to 5 (*Extremely*), measuring the extent to which different affective states have been experienced at a specific point in time. PA and NA reflect dispositional dimensions, with high NA characterized by subjective distress and unpleasant engagement, whereas PA refers to the extent to which an individual experiences pleasurable engagement with the environment. The PANAS is designed to measure affect in various contexts such as at present or in general. Since we were interested in measuring dispositional affect, we used the time frame 'in general'. The PANAS has good reliability and validity (61).

Dysfunctional Personality traits

The Dimensional Assessment of Personality Pathology - Short Form (DAPP-SF; (63,64) is the abbreviated version of the DAPP–BQ (Livesley & Jackson, 2009). The DAPP-SF has 136 items, scored on a 5-point Likert scale, assessing *DSM–IV* personality pathology. The four scales constitute the domains emotional dysregulation, dissocial behavior, inhibition and compulsivity. In this study, the DAPP-SF was not intended as an assessment of Axis-II diagnoses of psychopathology according to the DSM-IV criteria, but rather to assess maladaptive personality traits, with higher scores indicating a greater burden of maladaptive personality traits. In the context of our study design, i.e. exposure to acute psychosocial stress, we were explicitly interested in the mediating effects of emotional dysregulation and dissocial behavior on cognitive stress appraisal. Emotional dysregulation is a core feature indicating instability and is acknowledged as a more general personality dysfunction (65,66). This domain is organized around two core emotional traits, affective lability and anxiety, which are associated with cognitive disorganization, especially in times of stress. Dissocial Behavior pattern comprises callousness and rejection features which are also related to rigid cognitive style. Dissocial Behavior is also seen as an amplifier of the expression of other maladaptive traits (66).

Each scale of the DAPP-SF has a theoretical range from 1 to 5, with higher scores indicating greater personality pathology. The internal consistency of the DAPP-SF has been proven to be satisfactory (0.78–0.89) (63).

Psychological distress and childhood trauma

In order to exclude the potential effects of psychological distress and childhood trauma on the model, we administered The Brief Symptom Inventory (BSI) (67) and the Childhood Trauma Questionnaire-Short Form (CTQ-SF) (68,69).

The BSI is a 53-item self-report inventory in which participants rate the extent to which they have been bothered (0 ="not at all" to 4="extremely") during the past two weeks by various symptoms. The BSI has nine subscales (67). For this study,

we were interested only in the subscales Anxiety and Depression as indicators of psychological distress.

The CTQ-SF is a 28-item self-report questionnaire to assess the severity of multiple forms of abuse and neglect during childhood (68,69). The CTQ-SF measures five dimensions of childhood trauma: sexual abuse, physical abuse, emotional abuse, physical neglect and emotional neglect. Each item is rated on a five-point Likert-type scale ranging from (1= *Never true* to 5=*Very often true*). The questionnaire provides a score for each subscale (from 5 to 25) and a total score. For this study we used the total score.

Cognitive appraisal of acute stress

The Primary Appraisal Secondary Appraisal (PASA) scale is a self-report questionnaire based on the transactional stress model proposed by Lazarus and Folkman (1984). The 16-item PASA scale assesses the anticipatory cognitive appraisal of a stressful psychosocial situation using a six-point Likert scale (1=*Strongly disagree* to 6=*Strongly agree*).The scale has moderate to good internal consistency for both subscales (6).

The PASA scales are organized into Primary Appraisal and Secondary appraisal scales (70). Primary appraisal refers to a person's judgment about the significance of an event as stressful, demanding, or irrelevant. Secondary Appraisal assesses the available coping resources and options when faced with a stressor. Whereas high scores on the Primary Appraisal scale indicate that the situation is threatening or challenging for the individual, high scores on the Secondary Appraisal scale indicate that the individual has sufficient resources to handle the situation. Subjects were asked to complete the PASA scale during the anticipation period of the psychosocial stress task (TSST).

Trier Social Stress Test (TSST)

We applied the standard protocol of the TSST as described by Kirshbaum et al. (1993). Subjects were informed about the TSST procedure by the researcher and asked to prepare a 5-minute speech intended to convince a panel of judges regarding "why you would be a good candidate for your ideal job". Subjects were introduced to the panel of judges (2 persons), and subsequently given 5 minutes to prepare their speech while seated (Anticipation Period). Participants completed the self-report PASA scale at the end of the Anticipation Period. Next, the panel entered the room and the subjects were invited to stand and deliver their speech (Public Speaking Task). The Public Speaking Task was followed by a 5-minute Mental Arithmetic Task. During both tasks, the panel monitored the participants' performance without offering any verbal or non-verbal feedback, while maintaining affectively neutral facial expressions. After the task, the subjects were debriefed about the TSST.

STATISTICAL ANALYSIS

Data were checked for (multivariate) normality, linearity and multicollinearity. We did not observe outliers using Mahalanobis distance criterion (71). In 12 cases we observed missing values, representing <2% of all data. Cases with missing values were dropped list wise. T-tests were used to compare appraisal, attachment, affect and personality parameters between patients and healthy control women. Relationships between these variables were calculated using Pearson's bivariate correlations independently for patients and controls. Correlation coefficients for patients and healthy controls were compared using Fisher's z-tests.

We used structural equation modeling (SEM) analysis to test our model. The major advantages of this analysis are: a) the ability to identify direct and indirect effects, b) the ability to identify corresponding errors, c) to examine associations among multiple independent and dependent variables, and d) to test the invariance of the model independently within patient and healthy control groups. For the latter aim (d), we used a two-tier approach. The first tier was to analyze an unconstrained and a constrained model. In the unconstrained model, magnitude and significance of all direct and indirect paths from attachment and temperament to personality and stress appraisal were estimated independently in the patient and healthy control groups. The effects were free to differ across these two groups. In the constrained model, the effects of the independent and dependent variables were constrained to be equal across the patient and healthy control groups. If the constrained model significantly worsened the fit of the model in comparison to the unconstrained model, this would be evidence of differing relationships between the variables between groups (72). The second tier was to test whether personality variables contributed significantly to the quality of the model fit. For this aim, the magnitude of the direct paths to and from the personality variables were constrained to zero (i.e., no effect) in a series of models. We started with the most constrained model (all direct paths to and from personality parameters constrained to zero), and compared the fit to the model without constraints for the personality parameters. In subsequent steps, less constrained models were tested.

Model fit and path coefficients were estimated using a robust maximum likelihood (MLR) method to allow for deviation from multivariate normality and missing data (73). Nested models were compared using a chi-squared test with Satorra-Bentler correction, in which the degrees of freedom are equal to the difference in the degrees of freedom for the test-statistics of two models (74). Goodness of fit of the model was evaluated using a chi-squared statistic with non-significant p-value (P>0.05) and a χ^2/df ratio < 1.5, Comparative Fit Index (CFI) ≥ 0.95 (75), Tucker-Lewis Index (TLI) ≥ 0.95 (76), Root Mean Square Error of Approximation (RMSEA) < 0.06 (77), and a

Standardized Root Mean Square Residuals (SRMR) < 0.05 (78). Statistical significance of the path coefficients was established through the examination of the z-values (79).

Sensitivity analyses were conducted by dividing the sample into two subsamples: with and without anxiety or depressive symptoms (median split of the scores on the BSI subscales Anxiety and Depression). Furthermore, we also tested the invariance of the path model both overall and independently across women with and without a history of childhood trauma. All analyses were conducted using M-Plus version 7.31.

RESULTS

Sample description

Participants ranged in age from 18-45 years (M = 29.04 years, SD = 7.35), of which the patient and control groups were similarly aged (Table 1). Forty percent of the women were unmarried. The majority (83%) of the healthy controls were highly educated, whereas 55% of the patients had a high degree of education, 41% of the patients had a middle education degree. With regard to ethnicity, 94% of all participants were identified as Caucasian. Women with psychopathology reported significantly higher rates of childhood trauma and higher scores on depression and anxiety scales (Table 1). All women lived in the Rotterdam area of the Netherlands.

Univariate analyses

Descriptive statistics of the main study variables of both the patient and control groups are presented in Table 1. T-tests were used to compare the reported scores of cognitive appraisal, attachment styles, affective and dysfunctional personality traits (emotional dysregulation and dissocial behavior) between the patient and control groups. Significant differences were observed for every parameter examined: patients scored significantly higher on primary appraisal, but significantly lower on secondary appraisal of acute stress than healthy controls (Table 1). Furthermore, patients reported significantly higher scores on attachment related avoidance and anxiety dimensions than healthy women. Additionally, patients scored significantly higher on negative affect, but lower on positive affect dimensions than healthy women. Also, the scores on emotional dysregulation and dissocial behavior were significantly higher in patients than in healthy controls.

Bivariate correlations

Patients and healthy controls were further analyzed by calculating Pearson correlation coefficients independently per group. Moderate to large correlations were found among attachment insecurities (anxiety and avoidance), negative affect and maladap-

Table 1. Mean (SD) scores of all variables for the patient and control group, separately.

	Patients	**Healthy controls**	**t-test**
	Mean (SD)	*Mean (SD)*	
Age (years)	29.89 (7.69)	28.14 (6.91)	t(196)=-1.688; p=0.093
PASA scale			
Primary appraisal	9.24 (1.32)	8.26 (1.54)	t(193)=-4.788; p<0.001
Secondary appraisal	7.44 (1.19)	8.06 (1.06)	t(193)=-3.857; p<0.001
DAPP-SF			
Emotional dysregulation	212.36 (48.94)	135.16 (36.46)	t(184)=-12.374; p<0.001
Dissocial behavior	76.44 (19.64)	64.55 (16.32)	t(184)=-4.448; p<0.001
PANAS			
Positive affectivity	27.69 (7.69)	34.92 (5.61)	t(196)=7.515; p<0.001
Negative affectivity	31.76 (8.68)	18.63 (6.27)	t(196)=-12.144; p<0.001
ECR-r			
Anxious attachment	4.12 (1.24)	2.54 (1.15)	t(196)=-9.3320; p<0.001
Avoidant attachment	3.24 (1.11)	2.33 (0.84)	t(196)=-6.531; p<0.001
CTQ_{Total}	26.42 (8.17)	19.29 (5.57)	t(195) =-7.11; p<0.001
bsiANX	1.30 (0.98)	0.16 (0.23)	t(192) = -11.01; p<0.001
bsiDEP	1.52 (1.01)	0.21 (0.34)	t(192) = -12.04; p<0.001

Abbreviations: Primary Appraisal and Secondary Appraisal scale (PASA); The Dimensional Assessment of Personality psychopathology – short form (DAPP-SF); Positive and Negative affect scale (PANAS); Experience in Close Relationships- revised (ECR-r). The Childhood trauma questionnaire (total score) (CTQ_{Total}); The Brief Symptom Inventory, anxiety scale (bsiANX); The Brief Symptom Inventory, depression scale (bsiDEP).

tive traits (emotional disturbance and dissocial behavior) in both the healthy and the patient groups (Table 2). However, a moderate association between positive affect and secondary appraisal was found only in the healthy control group (Table 3).

Measurement model and model fit

The model fit was initially tested for the overall cohort, including both the patients and healthy control samples, in which the estimated path coefficients were unconstrained. The unconstrained model provided a good model fit: Chi2(2)=2.601; p=0.27;Chi2/df = 1.30; CFI=0.998; TLI =0.949; RMSEA = 0.055 (95%CI: 0.000 to 0.215); SRMR =0.020. Figures 1a and 1b show the final multi-group model for healthy controls and patients, respectively. In the final model, all path coefficients were constrained to be equal across the patients and healthy controls. The final model also provided a good model fit: Chi2 (24) =27.614; p=0.2766; Chi2/df = 1.15; CFI=0.989; TLI =0.974; RMSEA=0.039 (95% CI: .000 to .094); SRMR =0.085. Coefficients with 95% confidence

Table 2. Correlations between all variables, for the patient (top right corner, grey) and the control group (bottom left), separately.

	Primary appraisal	Secondary appraisal	Emotional dysregulation	Dissocial behavior	Positive affectivity	Negative affectivity	Anxious attachment	Avoidant attachment
Primary appraisal	1.00	-.25*	.17	-.00	-.11	-.03	.05	-.07
Secondary appraisal	-.30**	1.00	-.09	.03	.40**	-.01	-.02	-.01
Emotional dysregulation	.36**	-.05	1.00	.60**	-.18	.57**	.46**	.11
Dissocial behavior	.28**	-.06	.71**	1.00	-.06	.32**	.31**	.09
Positive affectivity	-.15	.38**	-.27*	-.16	1.00	-.06	-.11	-.09
Negative affectivity	.25*	-.16	.63**	.43**	.05	1.00	.29**	.01
Anxious attachment	.29**	-.14	.74**	.57**	-.13	.55**	1.00	.28**
Avoidant attachment	.14	-.15	.51**	.47**	-.19	.39**	.55**	1.00

*= p <0.01; **= p < 0.001

Table 3. Significance of differences in correlations between patients and healthy controls (Fisher Z-tests).

	Primary appraisal	Secondary appraisal	Emotional dysregulation	Dissocial behavior	Positive affectivity	Negative affectivity	Anxious attachment	Avoidant attachment
Primary appraisal	-	Z=-0.386 P=0.35	Z=1.364 P=0.086	Z=1.915 P=0.028*	Z=-2.861 P=0.002*	Z=4.035 P<0.001*	Z=1.758 P=0.039*	Z=1.444 P=0.074
Secondary appraisal		-	Z=0.274 P=0.392	Z=-.598 P=0.275	Z=-.187 P=0.426	Z=-1.04 P=0.149	Z=-.824 P=0.205	Z=1.045 P=0.148
Emotional dysregulation			-	Z=1.336 P=0.091	Z=-.607 P=0.272	Z=.617 P=0.269	Z=2.98 P=0.001*	Z=2.98 P=0.001*
Dissocial behavior				-	Z=-.007 P=0.497	Z=.824 P=0.205	Z=2.172 P=0.015*	Z=2.805 P=0.003*
Positive affectivity					-	Z=.783 P=0.217	Z=-.119 P=0.452	Z=-.715 P=0.237
Negative affectivity						-	Z=2.285 P=0.011*	Z=2.786 P=0.003*
Anxious attachment							-	Z=2.33 P=0.01*
Avoidant attachment								-

*: significant difference

intervals (CI) of the paths in the model are reported in Table 4. Thick lines represent significant paths (Figure 1). Dotted lines represent paths to and from the personality parameters that do not contribute to the model fit. The model demonstrated that in patients, attachment avoidance did not have direct or indirect effects on cognitive stress appraisal (β = -0.04, 95%CI = -0.14-0.23). Attachment anxiety was not directly associated with primary and secondary appraisal but had an indirect effect on primary appraisal outcome (β = 0.12, 95%CI = 0.03-0.21). With respect to the temperamental dimensions: positive affect was directly associated with secondary appraisal (β = 0.43, 95%CI = 0.31-0.56), whereas negative affectivity had an indirect effect on primary appraisal (β = 0.13, 95%CI = 0.03-0.23).

Notably, the model showed identical pathways in the healthy controls as observed among patients. Attachment avoidance exhibited no direct or indirect effects on cognitive stress appraisal (β = -0.03, 95%CI = -0.15-0.09). Attachment anxiety was not directly associated with primary and secondary appraisal, but it had an indirect effect on primary appraisal outcome (β = 0.01, 95%CI = 0.02-0.18). Positive affect was directly associated with secondary appraisal (β = 0.38, 95%CI = 0.24-0.51), while negative affect had an indirect effect on primary appraisal (β = 0.08, 95%CI = 0.01-0.14).

To understand the contribution of the dysfunctional personality trait parameters, paths to and from emotional dysregulation and dissocial behavior were fixed at zero in a stepwise procedure. Fixing the paths from dissocial behavior to primary and secondary appraisal at zero (model 7) did not result in a significant decrease of the model fit compared to the unconstrained model (model 0) (Table 5). In contrast, fixing paths to and from emotional dysregulation (models 1-3, and models 4-6) resulted in a significant worsening of the model fit, confirming the important contribution of emotional dysregulation to the quality of the model fit.

The final model accounted for 4% of the variance of primary appraisal and for 13% of the variance of secondary appraisal in healthy women. In patients, the model accounted for 10% of the variance of primary appraisal and 19% of the variance of secondary appraisal. The model also accounted for 60% of the variance of emotional dysregulation in healthy women and 46% of the variance of emotional dysregulation in patients.

Sensitivity analysis

Sensitivity analyses demonstrated an invariant model fit for women with and without anxious or depressive symptoms (Chi2(24)=30.589; p=0.167), and for women with and without a history of child abuse (Chi2(24) = 22.468; p=0.551). This indicates that the outcome of the model varies neither as a function of the presence of depressive or anxiety symptoms, nor the presence of a history of childhood trauma.

Table 4. Final model: Standardized direct and indirect effects and 95% confidence interval of attachment, temperament, and personality trait variables on primary and secondary cognitive appraisal, for the patient and control group separately.

	Healthy Control				Patients			
	Primary appraisal		**Secondary appraisal**		**Primary appraisal**		**Secondary appraisal**	
	Estimated	95%CI	Estimated	95%CI	Estimated	95%CI	Estimated	95%CI
Anxious attachment								
Direct effect	0.062	-0.101 to 0.225	-0.045	-0.223 to 0.091	0.073	-0.119 to 0.265	-0.040	-0.201 to 0.121
Indirect effects								
Anxious attachment -PA- Appraisal	0.004	-0.010 to 0.018	-0.031	-0.100 to 0.038	0.005	-0.011 to 0.021	-0.028	-0.089 to 0.033
Anxious attachment -NA - Appraisal	-0.035	-0.094 to 0.024	-0.048	-0.126 to 0.030	-0.042	-0.115 to 0.31	-0.043	-0.110 to 0.024
Anxious attachment - ED - Appraisal	0.101	0.019 to 0.183*	0.039	-0.059 to 0.137	0.120	0.026 to 0.214*	0.035	-0.051 to 0.121
Anxious attachment - DB - Appraisal	-0.020	-0.077 to 0.037	0.015	-0.042 to 0.072	-0.024	-0.093 to 0.045	0.013	-0.038 to 0.064
Anxious attachment - PA -ED - Appraisal	0.003	-0.005 to 0.011	0.001	-0.003 to 0.005	0.004	-0.006 to 0.014	0.001	-0.003 to 0.005
Anxious attachment - NA - ED - Appraisal	0.044	0.005 to 0.083*	0.017	-0.026 to 0.060	0.024	0.008 to 0.098*	0.015	-0.024 to 0.054
Anxious attachment - NA - DB - Appraisal	-0.005	-0.021 to 0.011	0.004	-0.012 to 0.020	-0.007	-0.025 to 0.011	0.004	-0.010 to 0.018
Anxious attachment - PA – DB - Appraisal	0.000	-0.002 to 0.002	0.000	-0.002 to 0.002	0.000	-0.002 to 0.002	0.000	-0.001 to 0.001
Total indirect	0.051	-0.000 to 0.001	-0.003	-0.123 to 0.117	0.109	0.005 to 0.213*	-0.003	-0.109 to 0.103
Total	0.153	-0.004 to 0.310	-0.047	-0.218 to 0.124	0.183	0.009 to 0.357*	-0.043	-0.196 to 0.110
Avoidant attachment								
Direct effect	-0.042	-0.160 to 0.076	-0.009	-0.148 to 0.130	-0.061	-0.235 to 0.113	-0.010	-0.163 to 0.143
Indirect effects								
Avoidant attachment - PA – Appraisal	0.006	-0.010 to 0.022	-0.047	-0.148 to 0.130	0.010	-0.014 to 0.034	-0.052	-0.115 to 0.011
Avoidant attachment - NA - Appraisal	-0.003	-0.015 to 0.009	-0.004	-0.020 to 0.012	-0.004	-0.022 to 0.014	-0.005	-0.023 to 0.013
Avoidant attachment - ED - Appraisal	0.005	-0.022 to 0.032	0.002	-0.010 to 0.014	0.008	-0.033 to 0.049	0.002	-0.010 to 0.014
Avoidant attachment - DB – Appraisal	-0.006	-0.076 to 0.044	0.004	-0.014 to 0.022	-0.008	-0.032 to 0.016	0.005	-0.015 to 0.025
Avoidant attachment PA – ED - Appraisal	0.005	-0.003 to 0.013	0.002	-0.004 to 0.008	0.008	-0.004 to 0.020	0.002	-0.004 to 0.008

Avoidant attachment - NA - ED- Appraisal	0.004	-0.012 to 0.020	0.001	-0.005 to 0.007	0.006	-0.016 to 0.028	0.002	-0.006 to 0.010
Avoidant attachment - PA - DB - Appraisal	0.000	-0.002 to 0.002	0.000	-0.002 to 0.002	-0.001	-0.003 to 0.001	0.000	-0.002 to 0.002
Avoidant attachment - NA -DB - Appraisal	0.000	-0.002 to 0.002	0.000	-0.002 to 0.002	-0.001	-0.005 to 0.003	0.000	-0.002 to 0.002
Total indirect	0.011	-0.026 to 0.048	-0.041	-0.098 to 0.016	0.017	-0.036 to 0.070	-0.045	-0.108 to 0.018
Total	-0.030	-0.153 to 0.093	-0.050	-0.194 to 0.112	-0.044	-0.138 to 0.226	-0.055	-0.226 to 0.116
Negative affect								
Direct effect	-0.072	-0.192 to 0.048	-0.098	-0.255 to 0.059	-0.118	-0.324 to 0.088	-0.121	-0.303 to 0.061
Indirect effects								
NA - ED - Appraisal	0.090	0.012 to 0.168*	0.034	-0.054 to 0.122	0.148	0.026 to 0.270*	0.043	-0.063 to 0.149
NA - DB - Appraisal	-0.011	-0.042 to 0.020	0.008	-0.023 to 0.039	-0.018	-0.069 to 0.033	0.010	-0.029 to 0.049
Total indirect	0.079	0.014 to 0.144*	0.043	-0.030 to 0.116	0.130	0.030 to 0.230*	0.053	-0.035 to 0.141
Total	0.007	-0.107 to 0.121	-0.055	-0.188 to 0.078	0.012	-0.172 to 0.196	-0.068	-0.229 to 0.093
Positive affect								
Direct effect	-0.052	-0.166 to 0.062	0.376	0.243 to 0.509***	-0.080	-0.252 to 0.092	0.433	0.306 to 0.560***
Indirect effects								
PA - ED - Appraisal	-0.043	-0.084 to -0.002*	-0.016	-0.059 to 0.027	-0.065	-0.124 to -0.006*	-0.019	-0.068 to 0.030
PA - DB - Appraisal	0.004	-0.008 to 0.016	-0.003	-0.015 to 0.009	0.005	-0.013 to 0.023	-0.003	-0.017 to 0.011
Total indirect	-0.039	-0.074 to -0.004*	-0.019	-0.058 to 0.020	-0.060	-0.111 to -0.009*	-0.022	-0.065 to 0.021
Total	-0.091	-0.201 to 0.019	0.357	0.237 to 0.477***	-0.139	-0.300 to 0.022	0.411	0.289 to 0.533***
Emotional dysregulation								
Direct effect	0.228	0.052 to 0.404*	0.087	-0.133 to 0.307	0.354	0.085 to 0.623*	0.102	-0.149 to 0.353
Dissocial behavior								
Direct effect	-0.059	-0.218 to 0.100	0.042	-0.121 to 0.205	-0.086	-0.319 to 0.147	0.047	-0.133 to 0.227

Abbreviations: Positive Affectivity (PA), Negative Affectivity (NA), Emotional Dysregulation (ED), Dissocial Behavior (DB);
* p<0.05; ** p<0.01; *** p<0.001

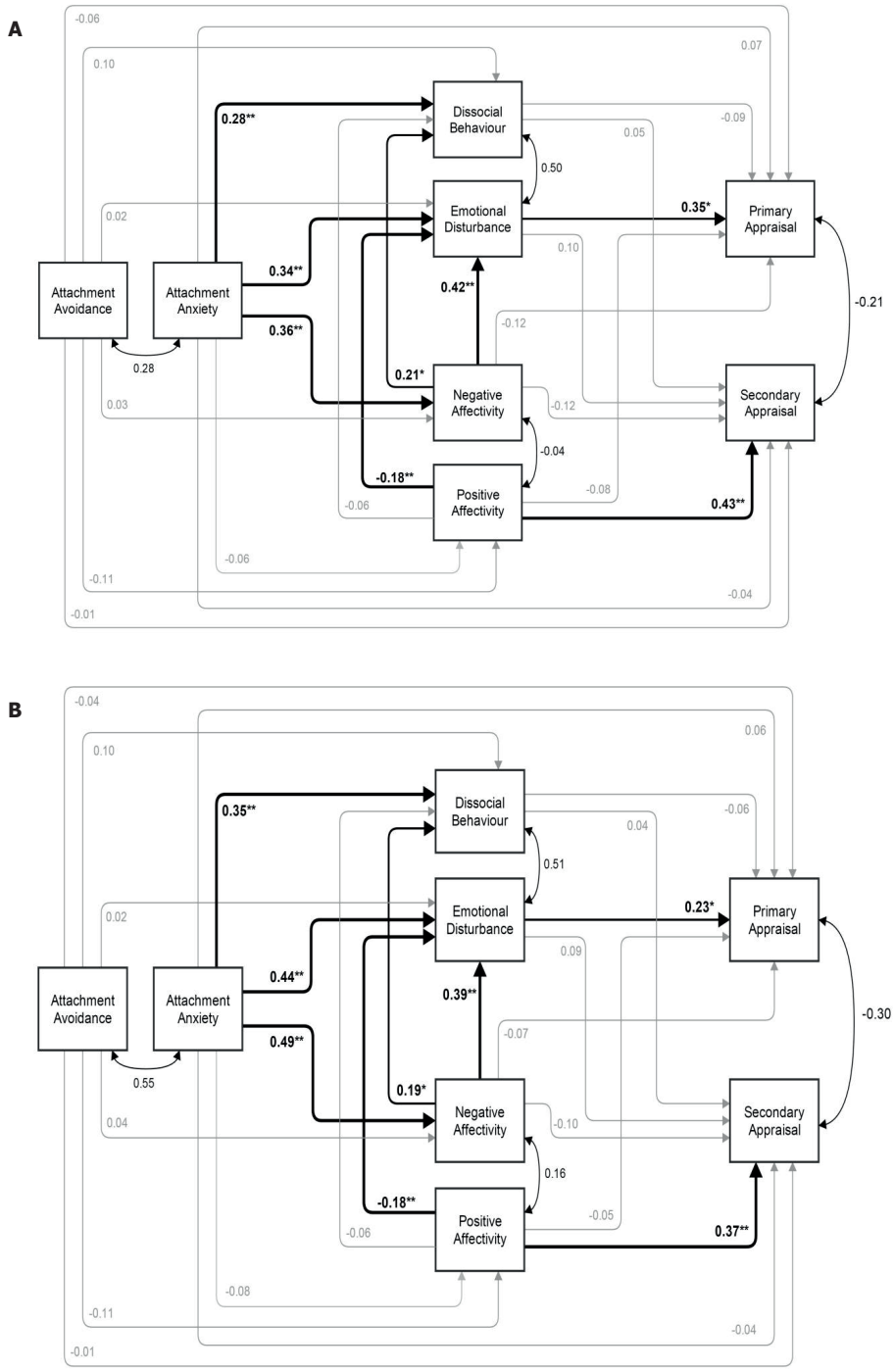

Figure 1. Path models and standardized path coefficients for the prediction of primary and secondary stress appraisal in the patient (A) and the healthy control (B) groups.

Table 5. Fit of the nested models with constraints for personality parameters.

Model	Description	Log-Likelihood	ΔChi-square[1]	Δdf	Significance
0	No constrains	-4244.11	-	-	-
Emotional dysregulation (ED) and Dissocial Behavior (DB)					
1	Paths from ED and DB to Primary and Secondary Appraisal constrained to '0'	-4249.50	10.90	4	P= 0.028
2	Paths to ED and DB constrained to '0'	-4364.33	180.90	8	P<0.001
3	All paths to and from ED and DB constrained to '0'	-4368.55	202.40	12	P<0.001
Emotional Dysregulation (ED)					
4	Paths from ED to Primary and Secondary Appraisal constrained to '0'	-4248.74	10.05	2	P<0.007
5	Paths to ED constrained to '0'	-4361.56	166.57	4	P<0.001
6	All paths to and from ED constrained to '0'	-4365.11	194.09	6	P<0.001
Dissocial Behavior (DB)					
7	Paths from DB to Primary and Secondary Appraisal constrained to '0'	-4244.56	0.85	2	P=0.654
8	Paths to DB constrained to '0'	-4276.07	60.50	4	P<0.001
9	All paths to and from DB constrained to '0'	-4276.56	61.50	6	P<0.001

[1] Change in Chi-square value compared to the unconstrained model including Santorra-Bentler correction

DISCUSSION

The aim of this study was to explore the role of general basic personality characteristics, such as adult attachment insecurity and temperament, on cognitive stress appraisals in a sample of women with both, high and low burden of personality psychopathology. We built a multifaceted model of cognitive stress appraisals that illustrates how adult attachment insecurities and temperament may operate in shaping the individuals' appraisals of psychosocial stress. Given that our sample consisted of healthy and females with high burden of personality psychopathology characterized with elevated emotional disturbance, we constructed our model with consideration of the mediating role of maladaptive personality features.

Although our model demonstrated no noticeable direct associations between attachment insecurities and cognitive stress appraisals, attachment anxiety was positively linked to negative affect and maladaptive personality traits. We found that emotional dysregulation was significantly associated with primary stress appraisal indicating that individuals with higher emotional dysregulation judge situations to be more stressful. Accordingly, our final model showed that the influence of attachment

anxiety and negative affect on primary cognitive appraisal of stress is mediated by dysfunctional personality traits. This finding suggests that attachment anxiety and negative affectivity do not fully capture the background of cognitive stress appraisal during psychosocial stress exposure. However, considering the strong association between attachment anxiety, negative affect and emotional disturbance (44,45,80) and their indirect link to cognitive appraisal, attachment anxiety and negative affectivity might be considered as predisposing factors for individual differences in cognitive stress appraisal.

The inclusion of individual components of dysfunctional personality traits uncovered the importance of emotional dysregulation in stress appraisal. This finding implies that, when exposed to a challenging situation, the significance of cognitive appraisal is augmented not only by varying influences of attachment style and/or temperament, but also by emotional dysregulation. Thus, when exposed unexpectedly to a socially threatening situation, someone with more affective susceptibility might be overwhelmed by intense emotion, leading to exaggerated perceptions of threat of a specific situation. In addition, our finding is also in line with studies suggesting that affective instability is a better predictor for the adaptive functioning than neuroticism (49). As such, this finding might be seen in the context of the multidimensionality of the mentalization approach, suggesting that under high arousal situations psychological cognitive understanding is relatively impaired and replaced by emotional automatic processing (81). It might be argued that this finding is particularly relevant in women with a higher burden of psychopathology who exhibit significantly higher levels of maladaptive traits such as emotional dysregulation (82).

Women with personality disorders reported significantly higher scores of attachment insecurity, higher levels of negative affectivity and lower scores of positive affectivity. However, the independent analyses in the healthy and clinical samples revealed identical paths underlying the mediating role of dysfunctional traits. From a clinical perspective, this finding supports the generally accepted approach that people in the general population typically exhibit a combination of adaptive and maladaptive personality traits. Recently, it was shown that even in people selected for having low levels of maladaptive traits, these traits were still associated with negative social integration and health outcomes (83). Although the importance of including dimensional scores of maladaptive traits in conceptual and empirical models of personality and health outcomes has been recently advocated in clinical studies, the majority of studies in healthy populations has failed to consider traits that do not fall within the normal range of personality traits. In addition, there is a significant lack of agreement and consistency in how maladaptive personality traits are assessed, defined and measured. In the present study, we used the DAPP-SF questionnaire to assess dysfunctional personality traits. Although the DAPP-SF questionnaire has been

introduced as a dimensional alternative to assess DSM-personality pathology, the DAPP-SF also shows meaningful relationships with normal personality traits (84). Our healthy controls were explicitly screened for a low burden of psychopathology and the absence of overt psychiatric illness. However, a substantial variability observed in dimensions in our healthy sample supports the argument that maladaptive personality features are not solely specific to clinical populations (85,86). Accordingly, maladaptive personality features should be considered more broadly in the general population as an approach to improving the understanding of the factors underlying adaptive responses to stress and long-term health outcomes.

Our model demonstrated that positive affectivity is directly linked to the secondary appraisal of psychosocial stress, and negatively associated with emotional dysregulation. In contrast, negative affectivity was not directly related to cognitive stress appraisal, however it was significantly associated with maladaptive personality traits. Therefore, individuals characterized with high positive affectivity appear to judge themselves as being more capable and having sufficient coping resources to negotiate stressful situations. This finding supports prior research indicating that positive affectivity provides a buffer against maladaptive responses to stress and to contribute to an individual's resilience (38,87). Earlier studies have demonstrated that resilient individuals who benefit from trait positive affectivity through stressful context reappraisal, tend to accomplish this using efficient emotional regulation and through more benign interpretations (87). Notably, the direct correlation between positive affectivity and secondary stress appraisal that we observed can be at least partly attributed to the incorporation of trait aspects of individual beliefs in one's ability and control expectancy within the PASA scales (6). However, positive affectivity was also negatively correlated with emotional dysregulation, which is considered to be a more state dependent maladaptive trait.

Sensitivity analyses demonstrated that the effects of childhood trauma and general distress did not impact the outcome of the model. Although a significant and long-lasting impact of childhood trauma on stress regulation has previously been postulated (88), correction for childhood trauma had no significant influence on the paths of the model. Similarly, the outcome of the model did not change as a function of anxiety or depression symptoms, which have been linked to elevated perceptions of threat (52).

Our finding that attachment insecurity and negative affectivity are not directly linked to cognitive stress appraisals may be specific to our research design as we triggered cognitive appraisal processes following a laboratory-based stress induction. Whereas attachment styles and temperament are considered stable patterns with their biological implications and lasting manifestation, cognitive appraisal is considered to be a dynamic process that might alter the perception of stress. In addition, it has been

proposed that individuals do not necessarily appraise acutely stressful situations in the same manner as less acute contexts (70). The advantage of the PASA scale is that this questionnaire assesses the cognitive appraisals of stress during an anticipation period, when the stressor is pending and individual's social-self is threatened. Moreover, the psychosocial stress task used in this study was not an attachment-related stressor which might have contributed to the lack of association between attachment style and cognitive appraisal. However, some studies have operationalized the effects of internal working models as support-seeking, self-esteem and self-worth (89). Therefore, the psychosocial stress paradigm which includes elements to intimidate an individual's self-worth (54), might consequently activate the attachment system.

The most notable limitation of this study is the modest sample size relative to typical recommendations for structural equation modeling. However, obtaining large samples of patients is difficult with a clinical assessment and detailed structured experimental protocol. Accordingly, our model will need replication in larger samples in order to confirm the validity and reliability of these findings. Furthermore, some caution should be used in interpreting the correlations between the constructs. For example, a high correlation between emotional dysregulation and negative affectivity might have contributed to the overlap between these constructs. However, although emotional dysregulation and negative affectivity are related, increasing evidence supports the conceptualization of emotion dysregulation as a distinct construct (50,90). Furthermore, structural equation modeling does not permit assessments of interaction effects on the outcome variable. Hence, we were not able to examine the interactive contributions of attachment and temperament on the cognitive appraisal, which might have better explained the individual susceptibilities to environmental demands.

This study makes several novel contributions to the existing literature. First, our model reveals that when exposed to a challenging situation, cognitive perception is augmented by the coincident influence of emotional dysregulation, and indirectly by varying influences of attachment style and temperament. Second, our model identifies that positive affect contributes to a buffering against maladaptive consequences of stress, which might be seen as a key to resilience. Lastly, we found that every observed association between attachment styles, temperament and cognitive stress appraisals, including the mediating role of maladaptive traits, applied equivalently to women with low or high burdens of psychopathology. Accordingly, this observation provides additional evidence that maladaptive personality traits are critical factors in understanding the contribution of individual characteristics on the cognitive appraisal of acute psychosocial stress.

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Chapter 3

No evidence for an interaction between 5-HTTLPR genotype and early life adversity on cortisol response to psychosocial stress in women

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ABSTRACT

The serotonin transporter gene-linked polymorphic region (5-HTTLPR) has previously been associated with hypothalamus-pituitary-adrenal (HPA) axis function. Moreover, it has been suggested that this association is moderated by an interaction with stressful life experiences. We investigated the moderation of cortisol response to psychosocial stress by 5-HTTLPR genotype, either directly or through an interaction with early life stress. One hundred and fifty one women performed the Trier Social Stress Test (TSST), during which salivary cortisol response patterns were assessed. Our results demonstrate a main effect of genotype on cortisol reactivity, in which women carrying two copies of the long version of the 5-HTTLPR exhibited stronger cortisol responses to psychosocial stress than women with at least one copy of the short allele. However, the proportion of the variance in cortisol response explained by 5-HTTLPR genotype as a single factor was not further strengthened when an interaction of 5-HTTLPR genotype with early life adversity was considered. Future studies are needed to further explore the psychophysiological and molecular factors affecting the relationship between 5-HTTLPR and HPA axis reactivity to psychosocial stress.

INTRODUCTION

Gene by environment interactions have been a widely touted, but often difficult to replicate, concept in psychiatric genetics (1). In particular, a considerable focus has been devoted to potential interactions between the serotonin transporter gene polymorphic region (5-HTTLR) and adverse life experience. A common 44 base pair insertion/deletion polymorphism in the 5-HTTLPR is known to be involved in the reuptake of serotonin by the serotonin transporter in the brain through transcriptional efficiency of the long (L) and short (S) alleles (2). The seminal report of a prospective longitudinal study of Caspi et al. (3), showing the S allele carriers to be more vulnerable to depression upon exposure to environmental adversities, was followed by many studies which varied in their success to replicate this finding (4-10). Furthermore, several meta-analyses, focused on the 5-HTTLPR by environmental stress interaction in depression as the outcome variable, demonstrated inconclusive results (11,12). Nevertheless, the diversity of studies and ongoing controversy have led to an increasing interest in stress-related biological pathways mediated by the serotonergic system in the development of psychopathology.

The hypothalamus-pituitary-adrenal (HPA) axis is one of most well studied mechanisms through which the 5-HTTLPR might interact with stressors (13). The serotonergic system has been suggested to be ideally positioned to regulate glucocorticoid secretion via its ability to influence neural activity at the hypothalamic, pituitary, and adrenal levels (12). Based on the observations of altered HPA axis activity in a broad range of stress-related psychiatric disorders (14,15), a number of studies have focused on the associations between the 5-HTTLPR genotype and HPA axis reactivity to acute stress (16-23). Thus far, contradictory results have been found. Several studies demonstrated that 5-HTTLPR homozygous S allele carriership is associated with elevated cortisol responsivity to psychosocial stress (16-18). However, other studies failed to support these initial findings (19-22), or reported opposite results (23). Recently, a meta-analysis has been published in which the authors reported statistically significant association of small effect between the 5-HTTLPR genotype and HPA axis reactivity to acute psychosocial stress with the SS variant demonstrating higher cortisol responses than the SL or LL variant of the 5-HTTLPR (24). In addition, there is increasing evidence that the association between 5-HTTLPR and HPA axis reactivity is stronger when stressful environmental factors are taken into account (18,22). Two previous studies have suggested that the effects of the 5-HTTLPR on cortisol reactivity are stronger in individuals with a history of stressful life events (19,23).

Taken together, the nature of the relationship between the 5-HTTLPR and cortisol reactivity remains unresolved. Our primary goal was to assess whether cortisol reactivity to psychosocial stress varies as a function of 5-HTTLPR genotype in a cohort

of women. In addition, we aimed to examine whether the magnitude of cortisol reactivity is modulated by an interaction between 5-HTTLPR genotype and childhood adversity. While a certain degree of challenge during childhood may enhance lifelong coping skills (25), overwhelming early life stress has been strongly associated with an increased lifetime risk of psychopathology (26). Therefore, in order to have the potential to evaluate a wider range of childhood maltreatment severity, we included both medication-free women who were recently diagnosed with personality disorder and at the beginning of outpatient therapy, as well as matched healthy controls.

METHODS

Participants

The study sample comprised 151 female participants of reproductive age (18-45 years). Women were self-referred in response to advertisements (n=66), or recruited from mental health outpatient clinics (n=85). Personality disorders were diagnosed using Axis II DSM-IV criteria (27). Patients were considered ineligible to participate if they had a medical or comorbid diagnosis of bipolar disorder, schizophrenia or current major depression. Women screened for the control group were excluded on the basis of any DSM-IV Axis I or Axis diagnosis, or any history of psychiatric or psychological treatment. In addition, global exclusion criteria for both groups included current medication (with the exception of oral monophasic contraceptives containing a combination of ethinylesatradiol and androgenic progestin), pregnancy, lactation, irregular menstrual cycle, and body mass index (BMI) < 18 or >30. In addition, women were excluded on the basis of any prior diagnosis of endometriosis, polycystic ovary disease, or gynaecologic infection. Naturally-cycling women were studied in the luteal phase of their menstrual cycle. Women using oral contraceptives were tested during the active pill weeks. The majority of the sample were Caucasian (n=139) and native Dutch speakers. Twelve Dutch-speaking women were of Netherlands Antilles heritage and mixed ethnicity.

Written informed consent was obtained from all the participants. The study was conducted according to the declaration of Helsinki and approved by the Medical Ethical Research Committee of the Erasmus MC, University Medical Center Rotterdam.

Procedure

After a structured interview by telephone to confirm the inclusion and exclusion criteria, participants were invited to the first session, which comprised the diagnostic interview for Axis I disorders using the Structured Clinical Interview for DSM-IV-TR

(SCID) (27). In addition, participants completed the Brief Symptom Inventory (BSI) (28-30) to evaluate psychological distress and psychiatric disorders, and the short form of the Childhood Trauma Questionnaire (CTQ) (31,32) to assess the severity of multiple forms of abuse and neglect.

The experimental session was scheduled during a second visit to the lab. The participants were asked to abstain from alcohol, nicotine, caffeine and intense physical activity for at least 24 hours prior to the experimental session. All measurements were performed between 14.00 and 16.00 hours to minimize potential circadian influences on cortisol responses. After an acclimatization period of 15 minutes, the experiment began with a baseline period of 5 minutes, after which a saliva sample was obtained. Subsequently, the participants underwent the TSST procedure. Immediately following the TSST, additional saliva samples were obtained at +1, +15, +35, and +55 minutes. The subjects were debriefed after the last saliva sample was collected.

Questionnaires

The Brief Symptom Inventory (BSI) (28-30) is a self-report questionnaire with 53 items on a four-point Likert scale assessing general psychological difficulties (total score) including somatisation, anxiety, depression, hostility (nine subscales). The BSI has adequate psychometric properties and good sensitivity to therapeutic changes.

The 28-item Childhood Trauma Questionnaire – Short Form (CTQ) was used to assess the severity of multiple forms of abuse and neglect during childhood (31,32) The CTQ has five domains: physical abuse, sexual abuse, emotional abuse, physical neglect, and emotional neglect. The total CTQ score was used as an index of childhood trauma.

The Trier Social Stress Test (TSST)

All participants performed the TSST. The TSST was administered according to the protocol of Kirshbaum et al. (33). First, the subjects were informed about the TSST procedure and asked to prepare a 5-minute speech intended to convince a panel of judges regarding why they would be a good candidate for their ideal job. Subjects were given 5 minutes to prepare their speech while being seated (Anticipation period). Next, the panel entered the room and subjects were invited to stand and deliver their speech (Public Speaking Task, PST). The PST was followed by a 5-minute Mental Arithmetic Task (MAT). During the PST and the MAT, the panel monitored the participants' performance without offering any verbal or non-verbal feedback, and while maintaining an affectively neutral facial expression. Furthermore, the subjects consented to audio-video recording of the session, for which the camera and tripod were positioned prominently within the room, in direct view of the subject.

Cortisol assay

Saliva samples were collected using Sarstedt Cortisol Salivette® cotton swab collection tubes. Participants were asked to chew on the swabs for 2 minutes to stimulate saliva flow. Samples were stored at -20 °C until analysis. The free salivary cortisol was measured using a commercially available ELISA kit (Demeditec Diagnostics, Kiel, Germany, DES6611). The inter- and intra-assay coefficients of variation were below 10% and 7%, respectively.

5-HTTLPR genotyping

DNA was isolated from the saliva collected with the Salivette® device from Sarstedt using an adapted version of the Qiagen Buccal Brush DNA purification kit. Purified DNA was PCR amplified using the following primers [Fw 5′-TGCGGGGGAATACTGGTAGG-3′; Rev 3′-GAACGTGGGAGGCAGCAGAC-5′]. Amplified DNA was separated with electrophoresis using a 2% agarose gel containing ethidium bromide. Electrophoresis was performed at 120V and 100mA for 2 hours. %-HTTLPR genotype was visually determined based on the height and number of DNA bands under ultraviolet light.

Statistical analysis

Statistical analyses were performed using the SPSS statistical software package (IBM SPSS Statistics, Version 21). Results are expressed as means ± SEM, unless otherwise specified. Data per parameter was tested for normality of the distribution using visual inspection of q-q plots and Levene's tests for homogeneity of variance. To meet the normality assumption, cortisol data was logarithmically transformed. For descriptive purposes, the mean data shown in the figures is presented in original units. Hardy–Weinberg equilibrium was determined based on the total 5-HTTLPR sample (N =151) using chi-square tests. Initial group comparisons between women with personality disorders and healthy controls were conducted using chi-square tests and analyses of variance (ANOVA). In subsequent analyses, main effects of, and interactions between, the 5-HTTLPR genotype (bi-allelic genotype classification) and CTQ total scores on the cortisol stress response were assessed. The cortisol response to the TSST was computed by subtracting the baseline measurement of cortisol from the peak value, 15 minutes after the stress test. In accordance with previous studies, we identified oral contraceptive status (non-users vs users) and psychopathology (healthy controls vs Cluster-C PD vs BPD) as variables associated with altered cortisol reactivity; these variables were entered as fixed factors in the analyses. Although equally distributed between genotypes (Table 1), additional analyses were calculated controlling for age, BMI and ethnicity to ensure robustness of the results. However, due to their insignificance, these variables were omitted from the final analyses. Greenhouse-Geisser

corrections were applied where appropriate, and adjusted results are reported. Effect sizes were calculated by partial eta squared (η^2). *P* values less than 0.05 were considered to be statistically significant.

RESULTS

Sample characteristics of the participants classified by 5-HTTLPR genotype are shown in Table 1. Participants were divided on the basis of the bi-allelic (SS, SL and LL) classification. Genotype frequencies were consistent with the Hardy–Weinberg Equilibrium [$\chi 2$ (1) = 0.43; *P* = 0.51]. Participants classified by 5-HTTLPR genotype did not differ regarding age, BMI, ethnicity, oral contraceptive use, distribution of psychopathology, childhood trauma score, or psychological distress score (all P-values $\geq$ 0.10).

We found a significant effect of the 5-HTTLPR genotype on cortisol responsivity to the TSST [$F(2,142) = 3.46$, $P = 0.03$, $\eta^2 = 0.05$]. Specifically, the LL allele carriers demonstrated the strongest cortisol responses to psychosocial stress (Figure 1). Analysis of covariance revealed no significant influence of the CTQ score [$F(1,44) = 0.07$, $P = 0.80$], nor was there a significant interaction of 5-HTTLPR x CTQ score on cortisol responsivity to the TSST [$F(2,142) = 0.66$, $P = 0.52$]. The main effect of 5-HTTLPR genotype remained significant when controlling for psychopathology and oral contraceptive use, as well as when age, BMI, and psychological distress were included as covariates. In addition, no effect of ethnicity was found on cortisol responsivity to the TSST. A significant main effect of psychopathology was observed [$F(2,141) =$

Table 1. Sample characteristics (mean, SD) categorized by 5-HTTLPR genotype.

	5-HTTLPR genotype				*P*
	Total *(n = 151)*	LL *(n = 46)*	SL *(n = 71)*	SS *(n = 34)*	
Age (*SD*)	28.29 ± 6.97	28.00 ± 6.03	28.51 ± 7.41	28.24 ± 7.38	0.93
BMI (*SD*)	23.13 ± 6.97	22.80 ± 3.20	22.98 ± 3.24	23.87 ± 5.12	0.41
Smokers No. (% yes)	23.2%	19.6%	18.3%	38.2%	0.10
Ethnicity No. (% Caucasian)	92.1%	89.1%	94.4%	91.2%	0.69
Oral contraceptives (% yes)	47.0%	41.0%	50.7%	47.1%	0.55
Psychopathology (% yes)	55.8%	56.5%	50.7%	67.6%	0.40
CTQ total score (*SD*)	43.54 ± 12.62	45.59 ± 13.41	41.52 ± 11.05	44.97 ± 14.25	0.18
BSI total score (*SD*)	0.77 ± 0.81	0.69 ± 0.59	0.70 ± 0.79	1.03 ± 0.81	0.10

Abbreviations: CTQ, Childhood Trauma Questionnaire; BSI, Brief Symptom Inventory; BMI, Body Mass Index.

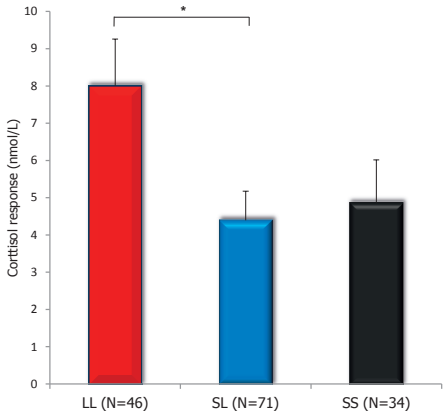

Figure 1. Mean (± SEM) salivary cortisol response to the Trier social stress test (computed by subtracting the baseline measurement time point from the peak value 15 min. post-stress) as a function of 5-HTTLPR genotype in female participants; (* $P < 0.05$).

3.28, $P = 0.04$, $\eta^2 = 0.05$], in which women with BPD exhibited significantly lower cortisol responses to the TSST. Moreover, we observed a significant main effect of oral contraceptives [$F(1, 141) = 12.82$, $P < 0.0001$, $\eta^2 = 0.08$] in which women using oral contraceptives had significantly lower cortisol responsivity to the TSST.

To ensure that the significance of the 5-HTTLPR effects on cortisol response were not influenced by sample stratification regarding psychopathology or contraceptive use, the relationship between the 5-HTTLPR genotype and cortisol responsivity to the TSST was examined separately for each psychopathology group as well as the healthy control group. Although comparisons within these subsamples lacked sufficient statistical power to established definitive conclusions, the qualitative genotype-dependent pattern of cortisol responsivity was similar in each group, with LL allele carriers showing the highest cortisol responses (Table 2). Likewise, a similar genotype-dependent pattern of cortisol response was observed as a function of oral contraceptive use, with LL allele carriers exhibiting higher cortisol responses to the TSST than SL or SS allele carriers (Table 2).

Table 2. Mean salivary cortisol response (SEM) to the TSST in each psychopathology and oral contraceptive group by 5-HTTLPR genotype

Cortisol response (nmol/L)	Psychopathology			Oral contraceptives	
	HC	Cluster-C	BPD	*Non-users*	*Users*
LL	9.40 (2.12)	8.45 (2.76)	5.40 (1.56)	9.80 (1.85)	5.45 (1.53)
SL	5.72 (1.20)	5.37 (1.74)	1.47 (1.09)	6.17 (1.27)	2.66 (0.88)
SS	4.22 (2.29)	8.01 (2.37)	2.44 (1.26)	6.76 (1.89)	2.74 (1.24)

Abbreviations: HC, Healthy Controls; BPD, Borderline Personality Disorder.

DISCUSSION

The results of our study demonstrated that 5-HTTLPR genotype is significantly associated with cortisol responsivity to psychosocial stress in women. In particular, women with the LL genotype demonstrated significantly higher free salivary cortisol responses to the TSST, compared to women carrying at least one S allele. Furthermore, the association of 5-HTTLPR genotype with cortisol responsivity to the TSST was not moderated by the burden of early life adversity as quantified by the CTQ.

While our results are in line with those of Mueller et al.(23), these findings are more difficult to reconcile with other studies (16-22). Sex-based influences might be one of the important sources of this distinction. Based on the existing literature, it is difficult to draw firm conclusions about the influence of sex with regard to associations between 5-HTTLPR and cortisol responses to stress. Most studies have failed to specifically address sex-based differences, mostly due to study design, inadequate power problems, and by modeling sex as a covariate. Two earlier studies observed that cortisol responsivity to stress was particularly enhanced in female homozygous S allele carriers (16,34). In contrast, we now report a significant, but opposite, association. Several reasons might be responsible for the differences between earlier studies and our study. First, there are notable differences in age. The majority of earlier studies investigated particularly young cohorts, including newborns and adolescents (16,18-21), whereas our study included adult females with a mean age of 28 years, in a period of reproductive hormonal cycling that is highly distinct from children and young adolescents. A broad range of different behaviors and effects on physiological systems are highly influenced by ovarian steroid functioning (35). In addition to the well-known effects of the menstrual cycle on HPA axis activity, several studies have suggested that ovarian steroids exert a strong influence on the serotonergic system (36-39). Therefore, the modulating effect of the 5-HTTLPR on the cortisol response to stress might be different across qualitatively distinct reproductive age cohorts. Accordingly, age accounts for a substantial proportion of the variance across 5-HTTLPR genetic association studies of depression (40,41).

The influence of hormonal contraceptives containing ethinylestradiol should also be noted. Considering that the majority of women during their reproductive age rely upon hormonal contraceptives, we ensured that we were adequately powered to examine the influence of oral contraceptives. Our inclusion criteria required that oral contraceptives contained the most commonly used preparation of ethinylestradiol in combination with androgenic progestins. We observed a main effect of oral contraceptive use on the cortisol response to psychosocial stress. Cortisol responsivity was significantly attenuated in women using oral contraceptives. This finding is consistent with the well-established estradiol-induced increase in CBG levels, thereby

enhancing the buffering capacity of serum cortisol with a reduction of free cortisol availability (42). Yet, this observed effect of oral contraceptive use did not alter the association between 5-HTTLPR genotype and cortisol responsivity to the TSST. For future studies, an even greater emphasis on endogenous and exogenous hormones is needed in order to identify the underlying mechanisms by which hormonal status influences HPA axis functioning in women and the relationship to the serotonergic system.

More than half of the women included in our study were diagnosed with a personality disorder and were seeking outpatient psychological treatment. Although our sample has notable distinctions from the majority of previously investigated samples, some of the clinical features such as stress-vulnerability are comparable to previous samples at high-risk for depression (16,34). Indeed, in this study we observed that altered HPA axis reactivity to stress was associated with psychopathology. We acknowledge that including women with psychopathology complicates the interpretation of the relationship between 5-HTTLPR genotype and cortisol reactivity. It has previously been suggested that psychopathological state affects both the HPA axis and the serotoninergic system (3,12,43,44). Nevertheless, when controlling for psychopathology status, the main effect of 5-HTTLPR genotype on the cortisol response to the TSST remained significant and in the same direction. Although we phenotyped 151 females, our sample was unfortunately underpowered to evaluate subsamples of psychopathological subgroups. However, inspection of these subsamples revealed a similar pattern of elevated cortisol reactivity in LL genotype carriers, compared to women with at least one S allele.

Regarding childhood trauma, our study conflicts with two earlier studies demonstrating that 5-HTTLPR genotype interacts with stressful life events in the cortisol response to psychosocial stress (19,23). However, it is important to note that adverse life events were assessed differentially between our study and these two earlier studies. Alexander et al., (19) demonstrated that homozygous S allele carriers had significantly higher cortisol responsivity, but this was only observed in people with a high burden of stressful life events. Mueller et al., (23) demonstrated that individuals carrying the LL allele showed a higher cortisol response to stress than S allele carriers, but this pattern was reversed when individuals were exposed to three or more stressful life events during the first five years of life. Our findings highlight the lack of a significant interaction between 5-HTTLPR genotype and burden of early life events on the cortisol response to psychosocial stress.

We employed the CTQ scale for the assessment of early adversity (31,32). The CTQ is a retrospective self-report inventory, intended to measure childhood abuse or neglect during the first 15 years of life. It is plausible that imprecise characterization of early adversities, type of incidence, and the time when this incidence has hap-

pened, might be a source of inconsistent findings. Unfortunately, we were not able to divide the CTQ score in more specific age periods of life, nor to define whether abuse was incidental or chronic. In addition, retrospective methods of assessment might result in impaired accuracy of answers due to recall bias. Nevertheless, good correlations have been reported between CTQ scores and clinician ratings obtained by semi-structured interviews (45). Furthermore, it has been suggested that sexual abuse and the 5-HTTLPR genotype have stronger effects on depressive symptoms than other forms of maltreatment (46). Therefore, subtypes of maltreatment may interact more specifically with genetic factors on HPA axis functioning and the etiology of stress-related disorders. Unfortunately, we did not have sufficient power in our sample to investigate different subtypes of childhood trauma, but a more detailed examination is needed and should be considered in future larger samples. However, stressful events occurring in childhood have been shown to be more consistently associated with neurobiological changes than those limited to adulthood (47,48). In addition, adversities in early childhood, compared to those limited to adulthood, have been demonstrated to interact with the 5-HTTLPR as a predictor of clinical depression (1).

The TSST has repeatedly been shown to be a reliable tool to elicit robust endocrine and cardiovascular responses in the vast majority of subjects (49). Notably, some studies which have utilized modified TSST protocols (e.g. by leaving out the presence of an evaluative audience) failed to observed an association between the 5-HTTLPR genotype and cortisol reactivity, suggesting the importance of the nature of the psychosocial stressor (19,20). However, several studies have demonstrated a significant impact of the 5-HTTLPR on cortisol response to a mild stressor in psychologically vulnerable subjects, which might suggest different relationships between genetic determinants and specific physiological and psychological processes (16,34).

Several limitations should be taken into account when evaluating our findings. We did not consider modulatory polymorphisms of the L allele, designated as L_A and $L_{G,}$ which have been reported to provide different levels of transporter expression (50-52). The L_G and S allele were demonstrated to have a similar serotonin transporter expression, both with lower expression than the L_A allele (50). However, other studies found no significant differences between classifications based on inferred levels of transporter expression (16,22). Our sample was also characterized by a minor ethnic heterogeneity, although women were primarily Caucasian (92.1%) and no significant differences were observed in the distribution of genotypes among ethnic groups. Although we enrolled a considerable sample of women (n=151 in total), it should be noted that the statistical power of our analyses was still relatively limited and, therefore, our study should be regarded as an exploratory study.

In conclusion, our findings support the notion that functional genetic variation is associated with cortisol responsivity to psychosocial stress. We observed that women

carrying the LL allele exhibit higher cortisol responses to psychosocial stress than women with at least one copy of the S allele. Our results reflect the need to clarify the sex-specific biological interaction between the serotonergic system and ovarian hormones, because these important factors are frequently overlooked. Furthermore, our results show that childhood maltreatment, specifically during the first 15 years of life, is unlikely to exert a modulating influence of 5-HTTLPR genotype on cortisol responsivity to psychosocial stress in women. Future studies are needed to clarify potentially contribution of biological and environmental factors, regarding the influence of 5-HTTLPR allelic variation on HPA axis reactivity to stress.

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Chapter 4

Borderline and Cluster C Personality Disorders Manifest Distinct Physiological Responses to Psychosocial Stress

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Psychoneuroendocrinology 2016; 72:131-138

ABSTRACT

Background. Maladaptive emotional control is a defining feature of personality disorders. Yet little is known about the underlying physiological dynamics of emotional reactivity to psychosocial stress across distinct personality disorders. The current study compared subjective emotional responses with autonomic nervous system and HPA axis physiological responses to psychosocial stress in women with cluster C personality disorder (CPD) and borderline personality disorder (BPD).

Methods. Subjective mood ratings, salivary cortisol, heart rate (HR), and skin conductance level (SCL) were assessed before, during, and after exposure to a standardized psychosocial stress paradigm (Trier Social Stress Test, TSST) in 26 women with BPD, 20 women with CPD, and 35 healthy female controls. Subjects were free of any medication including hormonal contraceptives, had a regular menstrual cycle, and were tested during the luteal phase of their menstrual cycle.

Results. Both CPD and BPD patients reported a similar burden of subjective mood disturbance. However, only BPD patients demonstrated reduced baseline cortisol levels with a blunted cortisol and HR reactivity to the TSST. In addition, BPD patients exhibited a generalized increase of SCL. No significant differences in baseline or TSST reactivity of cortisol, HR, or SCL were observed between CPD patients and healthy controls.

Conclusion. These findings indicate that patients with BPD have significant alterations in their physiological stress reactivity, which is notably distinct from patients with CPD and those of healthy controls.

INTRODUCTION

Individuals with personality disorders have significant difficulties in their interpersonal relationships, which has been widely attributed to dysfunction in affect regulation (1,2). Among the personality disorders, borderline personality disorder (BPD) is considered the prototype severe personality disorder (1,3). However, although clinically less disruptive, patients with Cluster C personality disorder (CPD) suffer from very comparable psychosocial impairment and subjective distress to that of BPD patients (4). Emotional dysregulation has been suggested to underlie many of the maladaptive behaviors, difficulties in the interpersonal domain, and dysfunctional coping strategies employed by patients with BPD (1,3,5,6). This maladaptive functioning across a broad range of personal and social situations is associated with a significant quality of life burden for individuals with BPD, resulting in an increased reliance on social support and health care services (7). A typical maladaptive interpersonal pattern is also identifiable in individual with CPD, who often experience dysfunctional relationships, hypersensitivity to negative evaluation (8,9), overregulation of emotions and impaired metacognition (10). Although CPD is presumed to be a less emotionally expressive disorder, adults diagnosed with CPD often exhibit heightened emotional reactivity (11).

Although BPD and CPD are equivalently common in clinical practice, BPD is by far the most intensively studied of the all personality disorder categories. This distinction is probably because of the relatively higher incidence of social rule-breaking behavior, suicide attempts, and self-injurious behavior in BPD patients which leads them to be more likely to have contact with mental health care providers. The wide range of behavioral problems in BPD requires a comprehensive treatment, whereas patients with cluster C personality disorder usually have a less problematic course in therapy (12). However, CPD patients also often remain in a passive patient role, demanding treatment without making the essential steps that are needed to recover. Therefore, cluster C personality disorders are also associated with high societal costs and low quality of life (13). Furthermore, although cluster C disorders (dependent and avoidant) are widely regarded as a disorder of medium severity, these assumptions have not been thoroughly studied in empirical studies.

The maladaptive emotional control of individuals with personality disorders has been hypothesized to result from childhood adversity and the quality of early-life attachment, most notably with neglecting or abusive primary caregivers (14–17). Such adverse events during early-life development may result in an insecure attachment style which in turn prevents the development of a proper affect regulatory capacity, impaired cognition, and coping in emotional relationships (15,18,19).

Despite an extensive literature on personality pathology and emotion regulation, the influence of personality features on the psychophysiological responses to psychosocial stress has not been sufficiently explored. In comparison with the most widely held models postulating HPA axis hyper-reactivity and reduced feedback sensitivity in BPD patients after neuroendocrine challenge testing (20,21), the few studies using psychosocial stress challenges have reported conflicting results (16,22–24).

Only four published studies to date have investigated HPA axis responsivity to psychosocial stress in patients with BPD. Simeon et al. (2007) found that a subgroup of patients with BPD and severe dissociation demonstrated a significantly higher peak cortisol reactivity to the Trier Social Stress Test (TSST) when compared to a less dissociative subgroup (24), suggesting that hyper-reactivity of the HPA axis is possibly the result of a symptomatic state rather than the trait of BPD. Nater et al. (2010) observed lower baseline cortisol levels and a blunted cortisol response to psychosocial stress in women with BPD compared to healthy controls (23). In line with these findings, Scott et al. (2013) demonstrated an attenuation of cortisol reactivity to the TSST in female BPD patients, although this may have resulted from elevated baseline cortisol levels or medication use (16). More recently, Deckers et al. (2015) reported significantly attenuated cortisol responses to psychosocial stress in BPD patients, however their use of a modified version of the TSST is difficult to compare with other studies (22).

Notably, the findings of previous studies of autonomic reactivity in BPD patients during psychosocial stress have not been entirely consistent. Depending upon the experimental conditions, BPD patients have been shown to exhibit autonomic hyperarousal (25) or the absence of autonomic modulation (24). However, more recent studies have presented a more consistent pattern of autonomic hypoarousal in patients with BPD (16,22,23). The divergence in the findings regarding autonomic nervous system responses in BPD is likely to be related to the multifactorial complexity of the stress response system, as well as the diversity of outcome measures employed.

Even less is known about this relationship in patients with CPD, although this cluster of personality disorders has been found to be among the most prevalent personality disorder in outpatient clinical populations and in the general community (26,27). Only one previous study has reported assessments of psychophysiological responses to stress in CPD patients (22), which found elevated subjective emotional reactivity similar to that of BPD patients, but without any discernible physiological differences compared to BPD patients and healthy controls. Thus, more empirical knowledge is needed to understand the relationship between emotional and physiological reactivity among the most highly prevalent personality disorders.

The current study was designed to compare emotional and physiological responses to psychosocial stress across three groups: outpatients with CPD, outpatients with BPD, and healthy controls. Importantly, our study design was chosen in an effort to

resolve some of the difficulties inherent in previous reports by integrating measurements of cortisol, HR, and SCL responses using the standardized version of the TSST. Moreover, all participants were enrolled and examined under strictly standardized conditions, including matching for age, body-mass index (BMI), medication, hormonal contraceptives, as well as the time of day and menstrual cycle phase during testing. Based on previous results, we hypothesized that BPD patients would exhibit elevated emotional distress with attenuated cortisol, HR, and SCL responses to the TSST, compared to healthy controls. We expected CPD patients to also report greater distress but with increased autonomic and HPA axis responses to the TSST, compared to healthy controls. In addition, considering high rates of insecure attachment style and childhood trauma in the patient samples, we also explored the impact of these early life adversities on psychophysiological responses to stress.

METHODS

Participants

The study was carried out at the department of Psychotherapy of the Riagg Rijnmond (Schiedam, The Netherlands) in collaboration with the department of Psychiatry of the Erasmus University Medical Center (Rotterdam, The Netherlands). Twenty-seven women diagnosed with BPD, 20 women with CPD, and 35 female healthy controls participated in the study. One of the 27 patients with BPD was unable to complete the testing procedure due to severe emotional reactions during the TSST. In total, data from 26 women with BPD, 20 women with CPD, and 35 healthy female controls were included in our analyses.

Patients were recruited from the outpatient mental health psychotherapy department of the Riagg Rijnmond. Diagnoses were made by experienced psychotherapists (psychiatrists, clinical psychologists and psychotherapists), based on DSM-IV Axis II criteria (28). In the BPD group, 16 women had co-morbid Axis II disorders, including avoidant (n = 7), dependent (n = 4), narcissistic (n = 3), and histrionic (n = 2) personality disorder. From a clinical perspective, the borderline presentation makes a critical difference in symptom expression between the comorbid clusters. Therefore, women with a diagnosis of BPD comorbid with avoidant, dependent, narcissistic, or histrionic personality disorder were classified in the BPD group and defined as having complex personality disorder (i.e., the patient meets the actual criteria for one or more personality disorders within more than one cluster). Participants with CPD, defined as having dependent and/or avoidant personality disorder without BPD, had no other Axis II co-morbidities and were therefore defined as having simple personality disorder (i.e., the patient meets actual criteria for one or more personality

disorders within the same cluster). No participants were identified with obsessive-compulsive personality disorder. Co-morbid Axis I diagnoses were assessed using the Structured Clinical Interview for DSM-IV axis I disorders (SCID-I) (Table 1) (28). Patients were considered ineligible to participate if they had a comorbid diagnosis of bipolar disorder, schizophrenia, current major depression, or used psychotropic medication within the previous 9 months. Healthy female controls were recruited from the community through local advertisements. Eligibility requirements included the absence of any DSM-IV Axis I or Axis II diagnoses, and no history of psychiatric or psychological treatment.

All subjects underwent a general medical examination prior to study enrollment. Exclusion criteria included: a) a history of any neurological or endocrine disorders, b) substance or alcohol abuse within the previous 4 months, c) BMI < 18, d) current pregnancy or lactation, and e) hormonal contraceptive use or irregular menstrual cycles within the previous 9 months.

Materials

Diagnostic assessments

Considering that dysfunctional personality traits are strongly associated with adversity and neglect during childhood, attachment quality and childhood maltreatment (1) were measured with self-report questionnaires.

The revised version of the Experiences in Close Relationships (ECR-r) is a self-report questionnaire with 36 items using a 7-point Likert scale for the assessment of attachment-related anxiety and avoidance (29,30). Participants were asked to think about their romantic partner while rating the appropriateness of each item on a 7-point Likert scale. For participants without a current partner, they were asked to rate how they feel generally during intimate relationships. Attachment-related anxiety and avoidance were dichotomized into high versus low using median split analysis [Anxiety: High (n = 36), Low (n = 38); Avoidance: High (n = 38), Low (n = 36)].

The 28-item Childhood Trauma Questionnaire – Short Form (CTQ) was used to assess the severity of multiple forms of abuse and neglect during childhood (31,32). The total CTQ score was dichotomized into high (n = 36) versus low trauma (n = 38) using median split analysis.

The Trier Social Stress Test (TSST)

The TSST was performed according to the protocol of Kirshbaum et al., 1993 (33). After an acclimatization period of 15 minutes, the experiment began with a baseline period of 5 minutes. Subsequently, the subjects were informed about the TSST procedure and asked to prepare a 5-minute speech intended to convince a panel of judges regarding "why you would be a good candidate for your ideal job". The subjects

were given 5 minutes to prepare their speech while seated (Anticipation Period). Next, the panel entered the room and the subjects were invited to stand and deliver their speech (Public Speaking Task, PST). The PST was followed by a 5-minute Mental Arithmetic Task (MAT). During the PST and MAT, the panel monitored the participants' performance without offering any verbal or non-verbal feedback, and maintaining an affectively neutral facial expression. Subjects provided written informed consent to allow audio-video recording of the session, for which a camera and tripod were positioned prominently within the room and in direct sight of the subject.

Subjective mood

To assess the subjective emotional state, we used the visual analogue scales (VAS) of the shortened 32-adjective Dutch version of the Profile of Mood States (POMS) (34,35). For each pair of adjectives, scores range from 0 to 100, defined by a mark placed by the subject on a standardized linear scale. The POMS measures 5 dimensions: depression, anger, fatigue, tension, and vigor. To compute the Total Mood Disturbance (TMD) score, the sum of the dimensional mean scores for depression, anger, fatigue and tension were subtracted from the dimensional mean score for vigor.

Procedure

The study was approved by the Medical Ethical Committee of the Erasmus University Medical Center. All participants provided written informed consent after the study procedures were fully explained both orally and in writing.

Participants were invited for two visits. During the initial visit, subjects completed questionnaires regarding their general medical health, severity of personality pathology, attachment style, childhood trauma, and subjective mood. Axis I co-morbidity was assessed by means of the SCID-I. During the second visit, the TSST was performed with continuous recordings of HR and SCL. Salivary samples for the assessment of cortisol were obtained 20 and 5 minutes prior to the TSST (baseline) and at +1, +15, +35, and +55 minutes after completion of the TSST. Changes in subjective mood were assessed by the POMS before, immediately following, and 45 minutes after the TSST. All measurements were performed between 14.00 and 16.00 hours to minimize circadian influences on the salivary and physiological assessments. Participants were asked to abstain from alcohol, nicotine, caffeine, and intense physical activity for at least 24 hours prior to the session, and to have been awake for at least 5 hours prior. Testing was performed during the luteal phase (day 20-28) of the menstrual cycle. Compliance with the instructions was assessed by means of a general checklist. Menstrual cycle phase was reconfirmed on the day of testing.

Hormonal and physiological measures

Saliva samples were collected using Sarstedt Cortisol Salivette® collection tubes and stored at -20 °C until they were analyzed. Free salivary cortisol was measured using a commercially available ELISA kit (Demeditec Diagnostics, Kiel, Germany, DES6611). The inter- and intra-assay coefficients of variation were below 10% and 7%, respectively.

Electrocardiographic (ECG) recordings were obtained using a precordial electrode, sampled at 512 Hz. SCL was assessed using 2 Ag/AgCl electrodes attached to the medial phalanx of the index and ring finger of the non-dominant hand and recorded at a sampling rate of 16 Hz. All data were stored on a flashcard by means of a portable digital recorder (Vitaport System®; TEMEC Instruments B.V., Kerkrade, The Netherlands). HR was determined from consecutive R-R intervals of the ECG. Recordings were visually inspected for detection and removal of artifacts. HR and SCL measurements were averaged across the baseline period and binned for each successive period of the TSST and recovery phase. Physiological responsivity to the TSST was determined as the difference between mean values during the baseline and TSST periods.

Statistical analysis

Statistical analyses were conducted using the SPSS statistical software package (IBM SPSS Statistics, Version 21). Results are expressed as mean ± SEM values unless otherwise indicated. *A priori* power analyses were performed with regard to cortisol level and HR. A total sample of n=78 participants for HR and n=69 for cortisol level would be required for detection of an interaction between time and condition of $\eta 2 \geq 0.15$ with a power of 0.80 at a significance level of $\alpha = 0.05$ (Cohen, 1988). The expected effect size of 0.15 is derived from the findings in previous studies examining cortisol (Deckers, 2015) and HR (Deckers, 2015; Weinberg, 2009). Effect size (η^2) was calculated from F values and degrees of freedom associated with the F-test (Nater et al., 2010).

Data was tested for normality of distribution using the Kolmogorov-Smirnov test, and for homogeneity of variance by visual inspection of the q-q plots and Levene's tests. To examine group differences in demographic and clinical characteristics, chi-squared tests (for categorical variables) and one-way ANOVAs (for continuous variables) with post-hoc testing (Scheffé) were conducted. In case of non-normality, the data was log transformed. Cortisol, HR, SCL, and subjective mood levels in response to the TSST were compared using a repeated measures analysis of variance (ANOVA), with Group (BPD, CPD, healthy controls) as a between-subject factor, and Time (baseline, TSST, recovery) as a within-subject factor. Confounding variables such as age, BMI, education, and smoking behaviour did not yield any significant main

or interaction effects on cortisol levels, HR, or SCL. For repeated measures ANOVA, the corresponding F values, degrees of freedom, and *p* values were corrected by the Greenhouse-Geisser procedure whenever the assumption of sphericity was violated. Effect sizes were calculated by partial eta squared (η^2). The value of eta squared ranges from 0 and 1. An eta squared <0.1 was interpreted as a weak effect, 0.1 to 0.3 as modest, 0.3 to 0.5 as moderate, and >0.5 as a strong effect. *P* values less than 0.05 were considered statistically significant. To reduce the possibility of a Type I error when analyzing stress reactivity, statistical significance for these tests was defined at the more stringent threshold of $P < 0.01$. Post-hoc analyses were carried out using Bonferroni adjustments. To control for the effects of the presence of post-traumatic stress disorder and eating disorders on cortisol responses, the primary analyses were re-run to serially exclude each of these diagnostic groups individually. Since the presence of Axis II psychopathology is strongly related with insecure attachment style, and childhood trauma, we performed sensitivity analyses in which we repeated the repeated measures ANOVA while including attachment style and childhood trauma as between-subject variables.

RESULTS

Subject characteristics

BPD, CPD, and healthy control (HC) groups were similar in age, BMI, smoking behavior, and educational attainment (Table 1). Patients with BPD reported higher childhood trauma CTQ scores than the other two groups ($P < 0.001$), for which no significant differences were found between CPD and HC. The ECR-r attachment anxiety score was highest for patients with BPD, intermediate for CPD, and lowest for HC (post-hoc analyses: BPD > CPD > HC, *p* values < 0.01) (Table 1). Similarly, patients with BPD showed higher ECR-r attachment avoidance score. No differences were found between patients with CPD and healthy controls.

Subjective mood

BPD and CPD groups had significantly greater mood disturbance (higher TMD scores) than the HC group at all time-points measured: baseline, TSST, and recovery period [Group: $F(2,77) = 18.52$; $p < 0.001$, $\eta^2 = 0.33$]. The TSST induced a time-dependent increase in TMD score [Time: $F(2,154) = 91.79$, $p < 0.001$, $\eta^2 = 0.54$] (Figure 1). A nominally significant Time x Group interaction was observed [$F(4,154) = 2.71$, $p = 0.04$]. Post-hoc analyses demonstrated that the increase in TMD elicited by the TSST was significantly elevated in BPD and CPD, compared to the HC [$F(2,79 = 3.52$, $p =$

Table 1. Subject characteristics, frequencies of Axis I diagnoses and clinical characteristics of the diagnostic groups

	BPD (n = 26)	CPD (n=20)	HC (n=35)	Statistics
Demographic data				
Age, mean (SD), years	29.23 (6.4)	31.35 (6.7)	28.60 (7.1)	$F(2,80) = 1.08$, $p = 0.35$
BMI, mean (SD), kg/m^2	24.50 (4.2)	23.49 (3.7)	22.41 (3.3)	$F(2,80) = 2.36$, $p = 0.10$
Smokers/ non-smokers (n)	9/17	6/14	8/27	$X^2(2,80) = 1.25$, $p = 0.54$
Educational level (n)				$X^2(2,80) = 4.98$, $p = 0.29$
Low	2	0	1	
Middle	13	7	11	
High	11	13	23	
Axis-I Diagnosis comorbidity				
Panic Disorder (n)	2	1	-	
Anxiety Disorder (n)	7	7	-	
Obsessive Compulsive Disorder (n)	4	3	-	
Eating Disorder (n)	12	6	-	
Post-Traumatic Stress Disorder (n)	7	1	-	
Psychometric data				
CTQ-SF, mean (SD)	56.2 (16.3)	42.5 (10.8)	39.5 (7.3)	$F(2,79) = 43.20$, $p < 0.001$
ECR-r, Attachment Anxiety, mean (SD)	4.7 (0.9)	3.7 (1.3)	2.6 (1.2)	$F(2,79) = 24.41$, $p < 0.001$
ECR-r, Attachment Avoidance, mean (SD)	3.6 (1.1)	2.9 (1.1)	2.4 (0.9)	$F(2,79) = 9.87$, $p < 0.001$

BPD: Borderline Personality Disorder; CPD: Cluster C Personality Disorder; HC: Healthy Controls
CTQ-SF: the Childhood Trauma Questionnaire – Short Form; ECR-r: the Experiences in Close Relationships-revised

0.04]. The BPD and CPD groups exhibited a similar heightened increase of the TMD score in response to the TSST.

Salivary Cortisol

The TSST induced a time-dependent increase in salivary cortisol levels [Time: $F(4,308) = 52.66$, $p < 0.001$, $\eta^2 = 0.41$], which differed significantly between the 3 groups [Group: $F(2,77) = 13.63$, $p < 0.001$, $\eta^2 = 0.26$] (Figure 2). Peak concentrations of post-TSST salivary cortisol levels were observed 15 minutes after completing the TSST. Patients with BPD demonstrated significantly lower baseline cortisol levels than either comparison group, with no differences observed between CPD and HC in baseline cortisol levels [$F(2,80) = 8.12$, $p < 0.001$, $\eta^2 = 0.17$; post-hoc analyses: HC (9.06 nmol/l) = CPD (11.63 nmol/l) > BPD (5.48 nmol/l)]. When baseline cortisol level was included as a covariate in the repeated measures ANOVA, a nominally

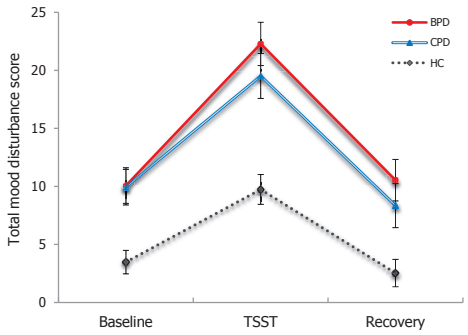

Figure 1. Subjective response to the TSST. The Total Mood Disturbance (TMD) score is presented as mean (±SEM) values in women with borderline personality disorder (BPD), cluster C personality disorder (CPD) and healthy controls (HC). The ANOVA for repeated measures demonstrated significant overall levels of mood disturbance between the groups ($p < 0.001$). The differences in response magnitude did not reach the statistical significance when the stringent criterion ($p<0.01$) was used (Group x Time, $p = 0.04$).

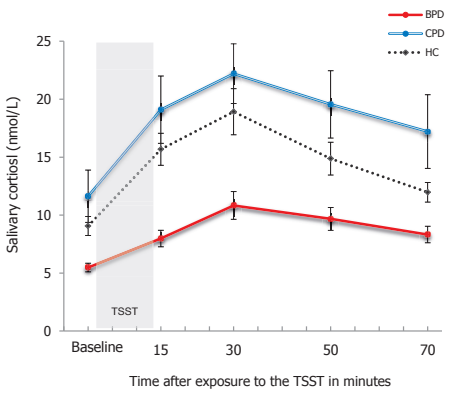

Figure 2. Salivary cortisol response to the TSST. Mean (±SEM) untransformed salivary cortisol values in women with borderline personality disorder (BPD), cluster C personality disorder (CPD) and healthy controls (HC). The ANOVA for repeated measures demonstrated significant differences in cortisol levels between groups in response to the TSST ($p < 0.001$).

significant Time x Group interaction was observed [$F(6,228) = 2.53$, $p = 0.04$]. Post-hoc analyses revealed that the BPD group had a blunted cortisol response to the TSST, in comparison to both CPD and HC. Although CPD patients had higher cortisol levels across all time points (Figure 2), no statistical differences were observed in the TSST-induced cortisol responses between patients with CPD and the HC group. Moreover, we evaluated whether BPD patients differed in their cortisol responses depending on the presence or absence of co-morbid CPD. BPD patients with co-morbid CPD (N=11) versus exhibited a highly similar cortisol response to the TSST compared to those without co-morbid CPD (N=14) [Group: $F(1,23) = 0.03$, $p = 0.86$].

Heart Rate

HR was significantly increased during the TSST, as demonstrated by a significant main effect of Time [$F(3,228)= 277,07$, $p < 0.001$, $\eta^2 = 0.79$], which varied in magnitude between the groups [Time x Group, $F(6,228) = 3.70$; $p < 0.01$, $\eta^2 = 0.09$]. The BPD group demonstrated a significantly blunted HR response to the TSST, compared to the CPD and HC groups [$F(2,78) = 4.49$, $p < 0.01$, $\eta^2 = 0.10$, post-hoc analyses: HC (increase 26.78 beats/min) = CPD (27.01 beat/min) > BPD (18.14 beats/min)] (Figure 3). Patients with CPD exhibited a similar HR response to the TSST as the HC group.

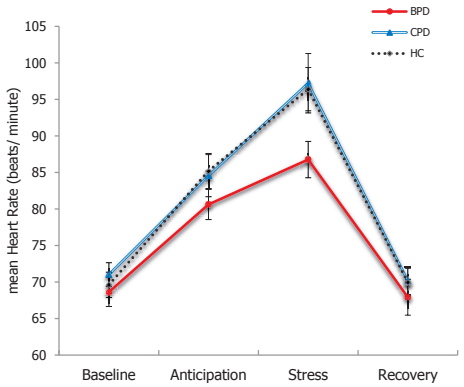

Figure 3. Heart Rate responsivity to the TSST. Mean Heart Rate is depicted as mean (±SEM) values in women with borderline personality disorder (BPD), cluster C personality disorder (CPD) and healthy controls (HC). The ANOVA for repeated measures demonstrated significant differences in response magnitude (group x time, $p< 0.05$) between the groups.

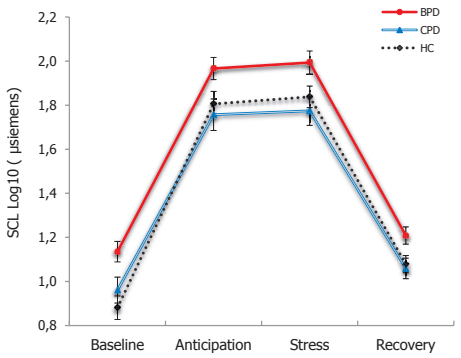

Figure 4. Skin conductance responsivity to the TSST. Log transformed skin conductance levels (SCL) are shown as mean (±SEM) values in women with borderline personality disorder (BPD), cluster C personality disorder (CPD) and healthy controls (HC). The ANOVA for repeated measures demonstrated significant differences in baseline SCL ($p< 0.01$). A significant main effect of group was found ($p< 0.01$) with BPD patients demonstrating higher overall SCL.

Skin conductance level

A significant main effect of Group was observed for the SCL measurements [$F(2,78) = 4.12$, $p < 0.01$, $\eta^2 = 0.10$), of which BPD patients exhibited an overall increase of SCL compared to both CPD and HC. The TSST induced a significant increase in SCL [Time: $F(3,231)= 1076.70$, $p < 0.001$, $\eta^2 = 0.93$], already evident during the anticipation period (Figure 4). When normalized for their respective baseline SCL, no significant difference in the SCL response to the TSST was observed between the groups [$F(2,79) = 2.74$, $p = 0.07$).

Sensitivity analyses

ANOVA analyses were repeated, excluding 1) patients with post-traumatic stress disorder, and 2) patients with an eating disorder. These analyses supported the direction and magnitude of the effects observed reported for the total sample, suggesting that the results cannot be explained by either comorbid post-traumatic stress disorder or eating disorders within our sample.

Additional sensitivity analyses revealed that participants reporting a higher level of childhood trauma has a significantly lower overall cortisol level [Group: $F(1,72) = 9.93$, $p < 0.01$, $\eta^2 = 0.12$]. Additionally, participants with the highest level of childhood trauma demonstrated significantly reduced HR responses to the TSST relative to

low-trauma participants [Time x Group: $F(3,71)= 3.55$, $p = 0.05$]. No significant main effects or interactions with childhood trauma were found regarding SCL.

Participants with higher levels of attachment anxiety exhibited blunted cortisol [Group: $F(1,72) = 4.39$, $p = 0.04$] and HR responses [Time x Group: $F(3,213) = 4.40$, $p = 0.02$] to the TSST. No significant main effects or interactions with attachment anxiety were found regarding SCL. No significant differences in cortisol levels, HR reactivity, or SCL responses were found between participants with high versus low levels of attachment-related avoidance. Taken together, these results confirm the relationship between Axis II psychopathology, insecure attachment, and childhood trauma.

DISCUSSION

This study was designed to investigate differences in affect regulation between female BPD and CPD outpatients in comparison to healthy controls by investigating HPA axis and autonomic responsivity to a well-established acute psychosocial stressor, the Trier Social Stress Test.

In response to the TSST, patients with CPD and BPD reported significantly higher subjective mood disturbance compared to healthy controls. Despite their similar subjective experience, BPD patients showed a highly distinct pattern of cortisol regulation: significantly reduced cortisol levels at baseline and a blunted response to the TSST. In contrast, CPD patients tended to have heightened cortisol levels and stress induced responses although this did not reach statistical significance, probably due to insufficient power. Furthermore, BPD patients demonstrated a blunted HR response to the TSST, whereas CPD patients and healthy controls had nearly identical HR responses. In contrast to the attenuated pattern of HR reactivity, the BPD group exhibited a significantly higher overall SCL, while SCL was similar between CPD patients and healthy controls. Additional analyses suggested that these results could not be explained by comorbid psychopathology such as post-traumatic stress disorder or eating disorders. Furthermore, in line with our expectations, we found that participants with higher levels of childhood trauma and/or increased attachment related anxiety exhibited attenuated cortisol and HR responses to the TSST, analogous to patient with BPD.

These findings underline the presence of a disturbed mood state and psychological hyper-reactivity among women with BPD and CPD, providing further support to the existing evidence of intense subjective negative affect in patients with severe personality pathology (20,36–38). High levels of negative affect may predispose individuals with Cluster B or C personality disorder to intense maladaptive emotional responses

and dysfunctional cognitive processes such as selective attention to negative or threatening cues (22,39,40) and hypervigilance to threat (41,42) Such maladaptive cognitive processes can result in emotional dysregulation and impaired social functioning (39). Moreover, an increased perception of threat accompanied by elevated emotional states might exacerbate negative responses to ongoing stressors or serve itself a source of chronic stress, with the resulting stress-related co-morbidities (43).

Our data demonstrate a contrasting pattern of cortisol responses to the TSST between CPD and BPD. Specifically, patients with BPD exhibited significantly lower baseline cortisol levels and a blunted cortisol response to the TSST, compared to either patients with CPD or healthy controls. This finding implies that independent of the presence of heightened mood disturbance in patients with personality disorder, the dysregulation of affect in patients with BPD is associated with a physiological hypo-reactivity, which appears specific for BPD compared to CPD. However, this finding should be considered with caution given the nominal statistical significance of the difference. Nevertheless, the finding of HPA axis hypo-reactivity in BPD patients replicates the earlier studies that reported blunted cortisol response to psychosocial stress in patients with BPD (16,22,23). It has been postulated that hypo-reactivity in BPD may result from the influence of early-life trauma due to chronic activation of the stress response system, including the HPA axis and autonomic nervous system (44). Although patients with CPD also report similar persistent psychosocial impairments and subjective distress (4), BPD and CPD patients differ significantly in their rates of exposure to harsh adversities in childhood and insecure attachment style.

Recent studies support the notion that attachment is biologically rooted (45,46). Early childhood attachment has a significant impact on the neurobiology of emotion regulation and psychosocial functioning in adulthood (45). However, an insecure attachment pattern in adulthood might be a predisposing factor that does not necessarily explain the psychophysiological features of personality disorders. Nevertheless, childhood trauma and deprivation of expected parental care have been previously associated blunted cortisol reactivity and the development of BPD (47–49). Moreover, there is growing evidence that adverse childhood experiences result in a lifelong blunting of HPA axis reactivity (47,50). Long-term modification of HPA axis function after childhood trauma exposure might be a homeostatic mechanism to protect against the detrimental effects of chronically elevated cortisol levels during sustained periods of stress in adulthood. Our results show similar effects of problematic attachment and childhood trauma on autonomic and HPA axis functioning in patients with BPD, but not in patients with CPD. However, given that our data is cross-sectional, we acknowledge the limitation of being unable to exclude reverse causality.

Our findings of a more pronounced overall SCL elevation in patients with BPD versus CPD or healthy controls in the presence of a blunted HR response to psycho-

social stress might be interpreted as an indication of a sympathetic / parasympathetic disbalance regarding stress reactivity in patients with BPD. Skin conductance level is an established index of sympathetic nervous system activity (51). Previous studies examining catecholaminergic responses to psychosocial stress failed to observe significant alterations of plasma epinephrine or norepinephrine responses in patients with BPD (23,24). Although we found an overall increase of SCL in patients with BPD patients, the observed responses to the TSST were notably similar across all three groups. Hence, the blunted HR response might be explained by an impairment of vagal withdrawal. Furthermore, our data are in agreement with studies reporting that early-life stress mediates diminished HR responses to stress in adulthood (50,52).

Although we controlled our study for relevant factors known to influence endocrine outcomes, the present findings should be considered in the light of some limitations. High rates of comorbid psychiatric diagnoses among patients with BPD, in particular post-traumatic stress disorder and eating disorders, might have confounded the observed outcomes. It has been previously reported that post-traumatic stress disorder and eating disorders are associated with alterations in HPA axis functioning (53–55). Importantly however, when patients with post-traumatic stress disorder and eating disorders were excluded from the analysis, our findings were not significantly altered. Moreover, it should be noted that a complex interaction of causal factors and comorbidities is highly consistent with personality disorders. Patients with BPD and CPD are often burdened with co-morbid psychiatric illnesses, such as eating disorders and/or post-traumatic stress disorder (55–59). Furthermore, when presuming childhood trauma as a causal factor of emotional dysregulation, BPD may be considered as a risk factor for post-traumatic stress disorder. Given the severity of emotional dysregulation, patients with BPD may have a higher likelihood of engaging in risky behaviors which could result in a consequently higher rate of exposure to potentially traumatic experiences. Unfortunately, our sub-samples were homogeneous in this respect and insufficient in size to explore whether the physiological responses are specific for BPD or might extend specifically to those patients with BPD who have a higher burden of childhood trauma or attachment insecurity. Future studies will be required in larger cohorts to identify additional risk and resilience factors that regulate the autonomic and HPA axis dysfunction in BPD versus CPD.

Taken together, our current findings demonstrate under highly controlled experimental conditions that unmedicated women suffering from either BPD or CPD exhibit analogous robust mood disturbances to psychosocial stress. However, patients with BPD demonstrated significant attenuations of cortisol and HR reactivity compared to patients with CPD or healthy controls. Moreover, this pattern of blunted cortisol and HR reactivity to psychosocial stress appears specific to patients with BPD, rather than simply a consequence of emotional vulnerability. In addition, our findings suggest

that the underlying physiological responses to stress among patients with BPD are not fully captured by subjective reporting of their emotional response, and thereby highlight the complexity of emotional dysregulation to psychosocial demands in patients with BPD versus CPD. A substantial proportion of CPD patients are known to function psychosocially at a qualitatively higher level than BPD patients (3). In our study, we found that CPD patients, in contrast with BPD patients, have a distinct psychophysiological responsivity to psychosocial stress, indicating a potentially distinct underlying biology. Furthermore, research on the relationship between childhood adversities, attachment insecurity, and HPA axis activity in personality disorders other than BPD remains sparse. Considering the high prevalence and burden of CPD, continued studies of Cluster C personality disorder is clearly warranted. Improved understanding of the psychophysiological responses across distinct personality disorders may help guide the development of novel psychotherapeutic or pharmacologic interventions to improve adaptive affective responses to psychosocial stressors and enhance quality of life.

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Chapter 5

The levonorgestrel-releasing intrauterine device potentiates stress reactivity

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ABSTRACT

Background. The levonorgestrel-releasing intrauterine device (LNG-IUD) is currently recommended as a first-line contraceptive with an exclusively local intrauterine influence. However, recent clinical trials have identified side effects of LNG-IUD that appear to be systemically mediated, including depressed mood and emotional lability.

Methods. We performed two experimental studies and a cross-sectional study. For each study, women were included from three groups: LNG-IUD (0.02mg / 24 hours), oral ethinylestradiol/levonorgestrel (0.03mg/0.15mg; EE30/LNG) and natural cycling (NC). Study 1 – Salivary cortisol was measured at baseline and at defined intervals following the Trier Social Stress Test (TSST). Heart rate was monitored continuously throughout the TSST. Study 2 – Salivary cortisol and serum total cortisol were evaluated relative to low-dose (1µg) adrenocorticotropic hormone (ACTH) administration. Study 3 – Hair cortisol was measured as a naturalistic measure of long-term cortisol exposure.

Results. Women using LNG-IUD had an exaggerated salivary cortisol response to the TSST (24.95 ± 13.45 nmol/L, 95% CI 17.49-32.40), compared to EE30/LNG (3.27 ± 2.83 nmol/L, 95% CI 1.71-4.84) and NC (10.85 ± 11.03 nmol/L, 95% CI 6.30-15.40) ($P < 0.0001$). Heart rate was significantly potentiated during the TSST in women using LNG-IUD ($P = 0.047$). In response to ACTH challenge, women using LNG-IUD and EE30/LNG had a blunted salivary cortisol response, compared to NC ($P < 0.0001$). Women using LNG-IUD had significantly elevated levels of hair cortisol compared to EE30/LNG or NC ($P < 0.0001$).

Conclusions: Our findings suggest that LNG-IUD contraception induces a centrally-mediated sensitization of both autonomic and hypothalamic-pituitary-adrenal (HPA) axis responsivity. LNG-IUD sensitization of HPA axis responsivity was observed acutely under standardized laboratory conditions, as well as chronically under naturalistic conditions.

INTRODUCTION

Since the launch of the first hormonal contraceptive in 1960, providing women with convenient and effective protection from pregnancy, continuous progress has been made in order to both minimize side effects and improve compliance without compromising efficacy (1). Long-acting reversible forms of contraceptives, such as the levonorgestrel-releasing intrauterine device (LNG-IUD), are currently among the most popular forms of birth control in North America and Europe (2-5). The National Institute for Health and Care Excellence and the American College of Obstetricians and Gynecologists both endorsed the use of the LNG-IUD as a first-line contraceptive option (6-8). This recommendation has also been advocated by the American Academy of Pediatrics, encouraging pediatricians to recommend the LNG-IUD to sexually active adolescents for prevention of unintended pregnancies (9).

In addition to providing long–acting protection (5 years per device), the LNG-IUD is a highly effective but rapidly reversible contraceptive method (10). Furthermore, the LNG-IUD can be used by women of any age or parity, requires minimal to no maintenance, has extensive evidence supporting its safety, and has an added value for a range of gynecological conditions (6,11-12). Moreover, and central to its popularity, the LNG-IUD is widely claimed to have no systemic physiological effects (13-15).

The most widely held model for the hormonal mechanism of action of LNG-IUD is by local intrauterine progestin release that results in extensive decidualization of the endometrium, an environment unsuitable for fertilization and implantation (10). The LNG-IUD has been shown to have little influence on ovarian activity, leading to widespread consensus that the release of LNG into the systemic circulation is below the physiologically-active level with a consequently negligible risk of adverse systemic effects (12,14-17). However, several studies have recently cast doubt upon the claim that LNG-IUD functions with an exclusively local intrauterine influence (18-20), due to side effects including depressed mood and emotional lability (20,21). A recent Danish population-based epidemiologic study established an association between progestin-containing hormonal contraceptives, including the LNG-IUD and other progestin-only contraception, with both a significantly elevated risk of diagnosis for depression and a higher rate of antidepressant use (22).

Although these findings might suggest a systemic influence of progestin release, direct physiological evidence has never been established. Extensive studies of baseline endocrine measurements have been performed without any significant alterations identified (23,24). However, baseline serum measurements might be insufficient to evaluate alterations in stress reactivity. Therefore, the aim of the current studies was to directly investigate whether the LNG-IUD influences the physiological responses to stress by examining autonomic and hypothalamic-pituitary-adrenal (HPA) axis

responsivity in women using the LNG-IUD, oral combination estrogen-progestin contraception, or naturally cycling. Moreover, to evaluate the possible influence of the IUD itself – independent of levonorgestrel – on cortisol responsivity during the TSST, we also recruited 10 women using a copper IUD.

In Study 1, we applied the Trier Social Stress Test (TSST) to induce moderate psychosocial stress in a laboratory setting. In Study 2, we performed the low-dose (1 μg) Synacthen test to distinguish between central and peripheral mechanisms of HPA axis functioning. In Study 3, we examined long-term cortisol exposure under naturalistic conditions using hair cortisol measurements.

MATERIALS AND METHODS

Study design and participants

The studies were approved by the Medical Ethical Committee of the Erasmus MC University Medical Center Rotterdam. The subjects were recruited from April 2011 to December 2013. All participants provided written informed consent after the study procedures were fully explained both orally and in writing. Subjects were recruited through posted flyers and local internet advertisements, and financially compensated for their participation. Inclusion criteria were female gender, age 18 to 45 years old, body mass index (BMI) between 19 and 30, and Dutch language fluency. All participants were assessed with a clinician-administered Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). Exclusion criteria were any Axis I psychiatric disorder (acute or in remission), current pregnancy or lactation, thyroid disorder, recent (within 4 months) medical illness, or use of any prescription medication other than hormonal contraceptives. Women having a prior diagnosis of endometriosis, polycystic ovary disease, or gynaecologic infection were excluded. In addition, women using hormonal contraceptives for treatment or prophylaxis of gynaecological (e.g. heavy menstrual bleeding) or dermatological (e.g. acne) conditions were excluded.

In each of the three studies, women were enrolled in the following groups: levonorgestrel-releasing intrauterine device (0.02mg/24hours; LNG-IUD, Mirena®), oral monophasic ethinylestradiol/ levonorgestrel (0.03mg/0.15mg; EE30/LNG) and natural cycling (NC). Women in the LNG-IUD and EE30/LNG groups were using these respective hormonal contraceptives for at least four months. Women in the LNG-IUD group with a regular menstrual cycle (25-33 days) were tested during the luteal phase (days 21-27). Women with amenorrhea secondary to the LNG-IUD were tested on a matched schedule with the other participants regarding the day of the week and time of day. Women in the EE30/LNG group were tested during the active pill weeks. The NC group consisted of women with a regular menstrual cycle, who

reported no use of hormonal contraceptives for at least four months. Women in the NC group were tested during the luteal phase of their menstrual cycle to minimize hormonal variation across the menstrual cycle. The luteal phase was determined by menstrual cycle tracking based on the length of each woman's menstrual cycle over the prior three months.

In addition to these three primary study groups, we also recruited 10 demographically-matched women using a non-hormonal copper-IUD, in order to evaluate the influence of the intrauterine device – independent of LNG – on stress responsivity during the TSST. Women using a copper-IUD were also tested during the luteal phase of their menstrual cycle.

The Positive and Negative Affect Scale (PANAS) was used to evaluate affective symptomatology (Table 1) (25). This scale consists of 20 adjectives describing a range of feelings and emotions, and measures general, positive and negative affective dimensions. Each item was rated on a 5-point Likert scale ranging from 1 (very slightly or not at all) to 5 (extremely) using the time frame 'in general'. The PANAS demonstrated high reliability (Positive affect: Cornbach's $\alpha = 0.89$, Negative affect: $\alpha = 0.85$) (25,26).

EXPERIMENTAL PROCEDURES

Study 1: Trier Social Stress Test (TSST)

In total, 55 healthy women participated in Study 1 (LNG-IUD, n=15; EE30/LNG, n=15; and NC group, n=25). The TSST was conducted according to the original protocol reported by Kirshbaum et al. (1993), including a preparation period, free speech task and verbal mental arithmetic task, each five minutes in duration (27). Subjects underwent the TSST in the presence of a two-member panel who maintained affectively neutral facial expressions throughout the procedure and provided the participant with no verbal or non-verbal feedback. Saliva samples were collected immediately prior (baseline) and at defined intervals following the TSST (+15, +30, +50, +70 minutes).

Heart rate (HR) was monitored continuously throughout the TSST procedure. Electrodes for electrocardiographic (ECG) signal recordings were applied using standard laboratory procedures, as previously described (28). Heart rate (HR) was determined using consecutive R-R intervals of the ECG and sampled at 512 Hz by means of a portable digital recorder (Vitaport System; TEMEC Instruments B.V., Kerkrade, The Netherlands). Interbeat intervals were calculated using R-top detection and visually inspected for detection and removal of artifacts. Physiological responsivity was evaluated by computing mean baseline-to-peak HR responses for each distinct phase of the TSST.

Testing was conducted using a highly standardized procedure. The TSST began 30 to 40 minutes after the arrival to the laboratory. Upon arrival to the lab, participants were administered a short interview of 10 to 15 minutes to allow them to feel at ease and to confirm their compliance with the instructions provided during the earlier pre-assessment. Thereafter, the physiological measurement procedure was explained and the electrodes were applied. Next, the participants were given a 10 minute period of quiet rest, seated in a room together with the experimenter, and permitted to read magazines and newspapers provided by the study staff. After the completion of the TSST, the participants remained seated in the same room as the experimenter for 55 minutes while again being permitted to read quietly. During this period, the experimenter interacted with the participants only for collection of the post-TSST saliva samples. At no time during the study procedure were participants permitted to use their cell phones, computers, or engage in any other activity. After collection of the final saliva sample, participants were fully debriefed about the study protocol.

Study 2: Low-dose (1µg) ACTH Stimulation Test

An entirely independent group of 60 healthy female participants were enrolled in Study 2 (LNG-IUD, n=20; EE30/LNG, n=20; and NC group, n=20). None of these women participated in Study 1. Adrenal cortex sensitivity was investigated using the low-dose (1µg) intravenous ACTH stimulation test (Synacthen®). Blood samples were obtained at baseline, +30, and +90 minutes following ACTH administration. Saliva samples were collected at baseline, +15, +30, +60, and +90 minutes. Baseline blood samples (2 x 9 mL) were obtained for assessment of corticosteroid binding globulin (CBG). Subjects rested quietly in a semi-recumbent position throughout the entire procedure.

In Studies 1 and 2, all measurements were performed between 14.00 and 16.00 hours in an effort to minimize any potential confounding of circadian influences. Participants abstained from alcohol, nicotine, caffeine, and intense physical activity for at least 24 hours prior to the experimental session.

Study 3: Naturalistic cortisol exposure

In total, 95 healthy women were enrolled in Study 3 (LNG-IUD, n=33; EE30/LNG, n=33; and NC group, n=29), of which 60 women (n=20 per group) also participated in Study 2. Approximately 150 hairs were removed with scissors at the posterior vertex position, as close to the scalp as possible. Hair samples were stored at room temperature in a paper envelope until analysis, secured and marked to indicate their proximal end. Cortisol measurements were performed using the most proximal 3 cm of the hair samples, corresponding to the cumulative systemic cortisol level during the prior three-month period (29).

Biochemical parameters

All samples were blinded upon collection to participant identity and group assignment using anonymized coding. Biochemical analyses were conducted by laboratory personnel who had no involvement or knowledge of the details of the sample collection. Saliva samples were collected using Sarstedt Cortisol Salivette® collection tubes and stored at -20°C until analysis. Salivary cortisol was measured using a commercially available ELISA kit (Demeditec Diagnostics, Kiel, Germany, DES6611). Inter- and intra-assay coefficients of variation were below 10% and 7%, respectively. Blood samples were immediately placed on ice upon collection and centrifuged for serum extraction. Serum was stored in aliquots at -80°C. Serum CBG was determined by radioimmunoassay (DRG Instruments GmbH, Marburg, Germany). Serum cortisol was measured using a two-site, solid-phase chemiluminescent immunometric assay (Immulite XPi, Siemens, Los Angeles, CA, USA).

Preparation and analysis of hair samples was performed as previously described (29). Fifteen milligrams of the most proximal 3 cm of hair was obtained for determination of the cortisol concentration. Extraction was performed in 1 mL of methanol at 52°C for 16 hours, evaporated under a constant nitrogen stream, and eluted into 250 µL of phosphate buffered saline (PBS, pH 8.0). Samples were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit for salivary cortisol (DRG Instruments GmbH, Marburg, Germany).

Statistical analysis

Results are expressed as mean ± SEM unless otherwise indicated. Data were tested for normality using the Kolmogorov-Smirnov Test and visual inspection of Q-Q plots. Homogeneity of variance was confirmed using Levene's test. To meet the normality assumption, all data were log transformed. To examine group differences in demographic characteristics and hair cortisol levels, chi-squared tests and one-way ANOVAs were conducted. Hormone profiles and HR responsivity during the TSST and ACTH stimulation test were compared using a repeated measures analysis of covariance (ANCOVA), with time as a within-subject factor and experimental group as a between-subject factor. For all general linear models, the corresponding F values, degrees of freedom and *P* values were corrected by the Greenhouse-Geisser procedure whenever the assumption of sphericity was violated. Effect sizes were calculated by partial eta squared (η^2). *P* values less than 0.05 were considered statistically significant. Post-hoc analyses were evaluated using a Bonferroni adjustment. Factors considered to be potentially confounding the main or interaction effects on cortisol and HR included: age, BMI, ethnicity, parity, PANAS scores, smoking, educational level, employment status, and duration of contraceptive use. These potential confounders were assessed in a set of ANOVAs with repeated measures. None of these

Table 1. Demographics and baseline characteristics

	LNG-IUD	EE30/LNG	NC	*P* value
Study 1: TSST	(n=15)	(n=15)	(n=25)	
Age, mean (SD), y	28.87 (7.43)	25.07 (5.98)	29.40 (5.75)	0.10
BMI, mean (SD), kg/m^2	21.11 (2.05)	21.82 (3.03)	22.82 (2.99)	0.17
Smokers/ non-smokers	0/15	2/13	4/21	0.29
Ethnicity: Caucasian/ non-Caucasian	10/5	14/1	22/3	0.11
Partner: yes/ no	6/9	4/11	12/13	0.41
Education: middle/high	4/11	3/12	9/16	0.54
Employment: labor/student	8/7	7/8	8/17	0.38
PANAS Positive Affect, mean (SD)	36.27 (6.8)	33.53 (6.37)	34.40 (6.11)	0.49
PANAS Negative Affect, mean (SD)	17.20 (6.41)	19.07 (8.08)	19.20 (7.26)	0.68
Heart Rate baseline, mean (SD), beats/min	67.42 (9.99)	73.19 (9.74)	70.41 (10.29)	0.33
Parous/ nulliparous	2/13	1/14	1/24	0.54
Duration of current contraception, median (IQR), months	15.0 (14.0)	47.0 (53.0)	-	-
Study 2: ACTH Stimulation Test	(n=20)	(n=20)	(n=20)	
Age, mean (SD), y	24.21 (4.28)	22.2 (1.47)	22.11 (2.85)	0.10
BMI, mean (SD), kg/m^2	22.6 (1.87)	22.53 (2.89)	21.66 (1.74)	0.22
Smokers/ non-smokers	2/18	1/19	2/18	0.80
Ethnicity: Caucasian/ non-Caucasian	16/4	18/2	17/3	0.68
Partner: yes/ no	11/9	8/12	11/9	0.62
Education: middle/high	5/15	4/16	7/13	0.55
Employment: labor/student	6/14	7/13	4/16	0.56
Parous/ nulliparous	0/20	0/20	0/20	-
Duration of current contraception, median (IQR), months	16.0 (12.0)	22.5 (42.0)	-	-
Study 3: Naturalistic cortisol exposure	(n=33)	(n=33)	(n=29)	
Age, mean (SD), y	24.94 (4.45)	23.15 (3.33)	23.59 (3.83)	0.16
BMI, mean (SD), kg/m^2	22.45 (1.90)	22.93 (2.55)	21.67 (2.20)	0.90
Smokers/ non-smokers	2/31	1/32	2/27	0.77
Ethnicity: Caucasian/ non-Caucasian	28/5	31/2	24/5	0.38
Partner: yes/ no	14//19	18/15	15/14	0.59
Education: middle/high	10/23	12/21	7/22	0.58
Employment: labor/student	9/24	7/26	10/19	0.51
Parous/ nulliparous	1/32	1/32	0/29	0.64
Duration of current contraception, median (IQR), months	18.0 (10.0)	25.0 (39.0)	-	-

Abbreviations: TSST (Trier Social Stress Test), BMI (Body Mass Index), PANAS (Positive Affect and Negative Affect Scale), ACTH (Adrenocorticotropic Hormone).

factors yielded significant main or interaction effects, on either cortisol levels or heart rate responses. Age and BMI were included as covariates in all relevant analyses. Additionally, considering that ethinylestradiol is known to influence CBG levels, for which a substantial proportion of CBG is bound to circulating cortisol, CBG concentrations were included as a covariate in the analyses of serum cortisol concentrations in Study 2. For all studies, *a priori* power analyses were performed to determine the required sample size at 80% power with a significance threshold of 0.05.

RESULTS

The flowcharts of study inclusion are shown in Figure 1. For each of the three studies, LNG-IUD, EE30/LNG, and NC groups had similar baseline characteristics, including age, BMI, smoking, and ethnicity (Table 1).

Study 1: Trier Social Stress Test (TSST)

The TSST induced a time-dependent increase in salivary cortisol ($F[1.87, 97.03] = 87.37$, $P < 0.0001$), which differed between the three groups ($F[2, 50] = 15.03$, $P < 0.0001$, $\eta^2 = 0.38$). A TSST x group interaction was observed ($F[(3.78, 94.42] = 11.84$, $P < 0.0001$, $\eta^2 = 0.32$; post-hoc analysis of peak cortisol response: LNG-IUD [24.95 ± 13.45 nmol/L, 95% CI 17.49–32.40] > NC [10.85 ± 11.03 nmol/L, 95% CI 6.30–15.40] > EE30/LNG [3.27 ± 2.83 nmol/L, 95% CI 1.71-4.84]) (Figure 2a).

Heart rate was increased during the TSST ($F[1.8, 93.44] = 201.77$, $P < 0.0001$), and varied in magnitude between the three groups (group, $F[2, 52] = 3.79$, $P = 0.03$; group x time, $F[3.59, 93.44] = 2.60$, $P = 0.047$; post-hoc analysis: LNG-IUD > EE30/LNG = NC). In particular, women using LNG-IUD demonstrated a strong potentiation of HR response, compared to women using EE30/LNG or NC (peak HR response: LNG-IUD [38.56 ± 18.14 beats/min, CI 95% 28.51–48.61] > EE30/LNG [28.24 ± 15.07 beats/min, CI 95% 19.89–36.58] = NC [27.57 ± 12.41 beats/min, CI 95% 22.45–32.69]) (Figure 2b).

Importantly, women using a copper-IUD had similar cortisol responses (peak cortisol response = 6.49 ± 5.70 nmol/L, CI 95% 2.41–10.57) to the NC group ($F[1, 31] = 3.41$, $P = 0.08$). Furthermore, no difference in heart rate response was observed between women using a copper-IUD (peak HR response = 28.35 ± 5.70 beats/min, CI 95% 24.27–32.43) versus the NC group ($F[1, 31] = 0.05$, $P = 0.80$).

Study 2: Low-dose (1µg) ACTH Stimulation Test

Administration of low-dose (1µg) ACTH induced a time-dependent increase in salivary cortisol ($F[2.31,131.75] = 356.66$, $P < 0.0001$), which differed by group (F[2,

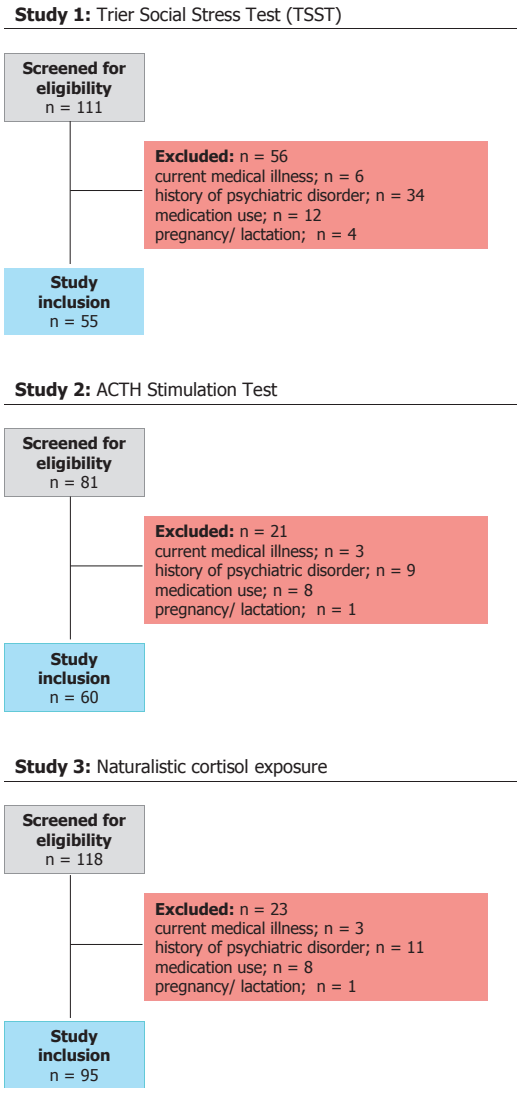

Figure 1. Flowchart of participants.

55] = 7.18, $P = 0.002$, $\eta^2 = 0.21$) during the post-administration period (group x time, F[4.67,128.55] = 8.22, $P < 0.0001$, $\eta^2 = 0.23$; post-hoc analysis: NC > LNG-IUD = EE30/LNG, $P = 0.001$) (Figure 3).

ACTH induced an increase in total serum cortisol (F[2, 114] = 373.08, $P < 0.0001$). The groups differed significantly with the EE30/LNG group displaying a significantly higher total serum cortisol response in comparison with NC or LNG-IUD groups (F[2, 57] = 65.59, $P < 0.0001$). A significant Group x Time interaction was observed (F[4,114] = 9.76, $P < 0.0001$, $\eta^2 = 0.69$). Corticosteroid-binding globulin (CBG) levels

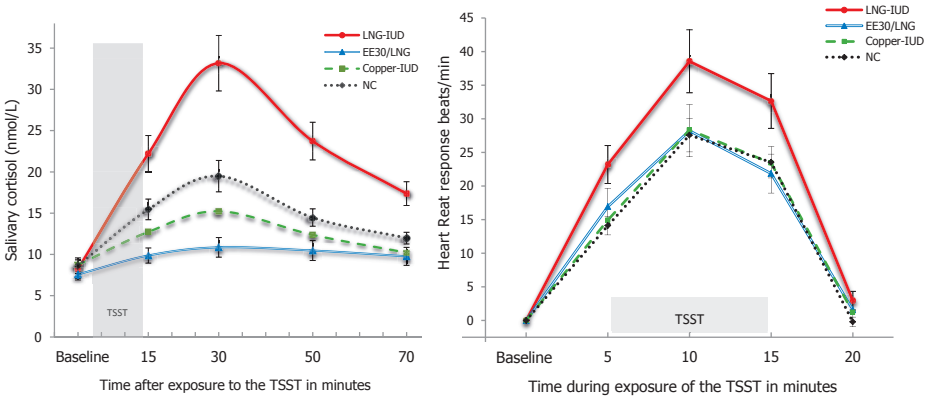

Figure 2. Salivary cortisol and Heart rate response to the TSST
A. Untransformed raw cortisol mean (±SEM) values in women using LNG-IUD, EE30/LNG or Copper-IUD, and naturally cycling women. ANCOVA demonstrated significant differences in cortisol between groups in response to the TSST (group, $P < 0.0001$; group x time, $P < 0.0001$). **B.** Heart rate responses are reported as mean (±SEM). ANCOVA demonstrated significant differences in response magnitude (group, $P = 0.03$; group x time, $P = 0.047$).

differed significantly between the groups (F(2,59) = 143,34; P < 0.001, η2= 0.83) and were positively correlated with serum cortisol at baseline ($r = 0.82$, $P < 0.001$) and at the +30 min post-ACTH peak ($r = 0.93$, $P < 0.001$) (Figure 5). Notably, after controlling for CBG levels, no significant group or interaction effect on total serum cortisol remained. In addition, both the serum and salivary cortisol findings remained significant despite correction for all known glucocorticoid receptor (*N3RC1*) poly-

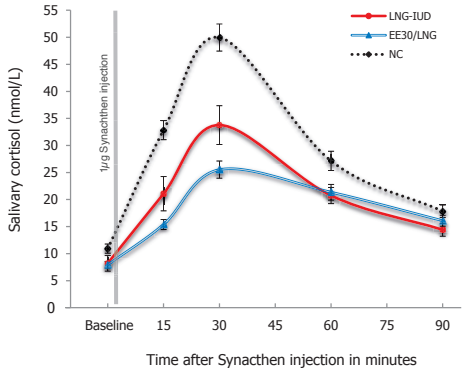

Figure 3. Salivary cortisol response to a low dose (1µg) ACTH stimulation
Untransformed raw cortisol mean (±SEM) values in women using LNG-IUD or EE30/LNG, and naturally cycling (NC) women. ANCOVA demonstrated significant differences in cortisol between groups following low-dose ACTH (group, $P = 0.002$; group x time, $P < 0.0001$).

morphisms that have been previously shown to modulate cortisol levels: rs6189/rs6190, rs6195, rs6198, rs10052957, rs41423247 ($P < 0.001$) (30).

Study 3: Naturalistic cortisol exposure

Hair cortisol differed significantly between groups ($F[2,90] = 13.35$, $P < 0.0001$). Analogous to the findings of the TSST, women using LNG-IUD had elevated hair cortisol and EE30/LNG users had reduced hair cortisol, compared to NC women (post-hoc analyses: LNG-IUD > NC > EE30/LNG, $P = 0.047$) (Figure 5).

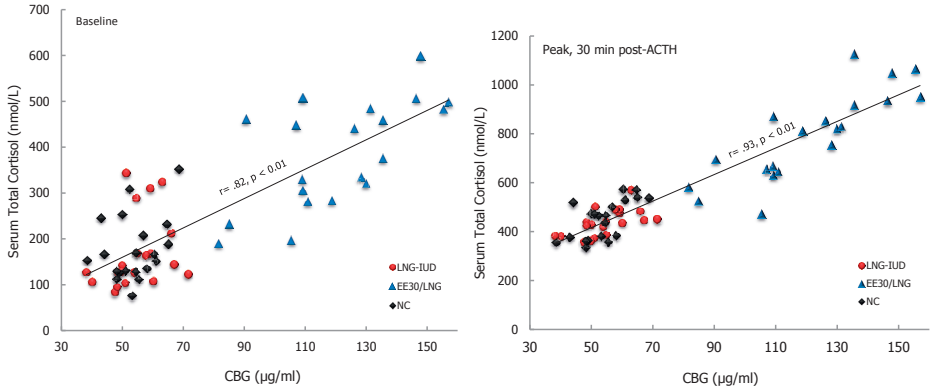

Figure 4. Correlation between CBG and serum total cortisol. Pearson correlation index in total group (**a**) baseline (r = .82, $P < 0.001$) and (**b**) peak (r = .93, $P < 0.001$). Abbreviations: CBG (Corticosteroid Binding Globulin).

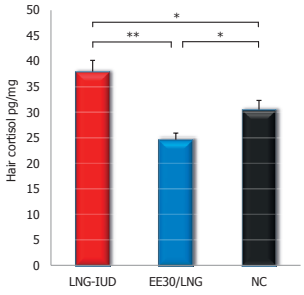

Figure 5. Hair cortisol concentrations. Untransformed hair cortisol mean (±SEM) values in women using LNG-IUD or EE30/LNG and naturally cycling (NC) women. ANCOVA demonstrated significant differences in hair cortisol between groups ($P < 0.001$). * $P < 0.05$, ** $P < 0.001$ calculated using ANOVA.

DISCUSSION

These studies are the first to demonstrate that the LNG-IUD alters the systemic physiological responses to stress. In particular, we find that women using LNG-IUD have substantially potentiated free cortisol and heart rate responses during moderate psychosocial stress compared to oral estrogen-progestin contraception or natural cycling.

Our data demonstrate a contrasting pattern of hormonal contraceptive modulation of endocrine responses to the TSST. Relative to natural cycling, women using LNG-IUD exhibited a robust potentiation of the salivary cortisol response during the TSST, whereas women using combination estrogen-progestin contraception showed a relatively blunted cortisol response. Ethinyl estradiol has been previously shown to increase CBG levels by approximately two-fold, thereby significantly enhancing the buffering capacity of serum cortisol with a concomitant reduction of the unbound fraction (31-33). In addition to a potentiation of cortisol responsivity, women using LNG-IUD had a >10 beats/min increase of psychosocial stress-induced heart rate. In contrast, copper-IUD users had cortisol and HR responses that were similar to naturally cycling women, confirming that secreted progestin was responsible for the potentiated stress responsivity in women using the LNG-IUD.

Mechanistically, we examined whether the observed changes in cortisol responsivity during the TSST were due to central (hypothalamic/pituitary) versus peripheral (adrenal cortex/CBG) alterations. In women using combination estrogen-progestin contraception, direct stimulation of the adrenal cortex using low-dose ACTH stimulation resulted in a blunted salivary cortisol response, analogous to the outcome following the TSST. In contrast, the blunted salivary cortisol response to ACTH in women using LNG-IUD, which led to a potentiated cortisol response during the TSST, occurred despite a normal CBG level. Taken together, these findings suggest a homeostatic downregulation of adrenal cortex function in LNG-IUD users secondary to the chronic potentiation of acute cortisol responsivity. Therefore, LNG-IUD appears to induce both a centrally-mediated potentiation of HPA reactivity and a peripheral downregulation of adrenal cortex reactivity.

We also investigated whether the alterations identified using the laboratory-based assessments of acute HPA axis responsivity were evident in measurements of longitudinal cortisol levels. Previous studies have established the reliability, sensitivity, and validity of hair cortisol measures for longitudinal assessments (34). Therefore, we sought to determine the real-world relevance of the observed laboratory-based findings by sampling hair cortisol in women under naturalistic conditions. Indeed, similar to the findings of the TSST, women using LNG-IUD showed significantly higher concentrations of hair cortisol than naturally cycling women. Conversely,

women using combination estrogen-progestin contraception had decreased hair cortisol levels, again reflecting the changes observed during the TSST. Together, these data confirm the naturalistic relevance of the influence of LNG-IUD on chronic HPA axis functioning.

The present study was not possible to implement using randomized group allocation due to ethical considerations in designing studies to investigate medication side effects (35). However, non-randomized designs are well suited for the study of unintended pharmacological effects (36). Moreover, we made extensive efforts to control for potential sources of bias. First, we established strict definitions for each contraceptive group. Second, we considered multiple potentially confounding variables including age, BMI, ethnicity, affective symptoms, duration of contraceptive use, parity, education, employment status, and smoking. Third, we attempted to replicate the main effect of LNG-IUD in both an experimental study using controlled laboratory conditions and an observational study under naturalistic conditions.

We observed potentiated cortisol responsivity (Studies 1 and 3) in the setting of a downregulation of adrenal cortex function (Study 2) in healthy women using the LNG-IUD. Elevated basal cortisol levels and HPA negative feedback dysregulation have been consistently linked to affective symptomatology. Alterations in HPA axis responses are present in a significant proportion of people with affective disorders (37). Moreover, previous studies have shown that women of childbearing age exhibit demonstrable HPA axis and mood alterations in response to sex steroids (38-40). Unfortunately, the design of our study did not permit the determination of sex steroid levels that might have helped to further elucidate the underlying mechanism by which the LNG-IUD modulates stress responsivity. We further acknowledge that our study does not permit an assessment of whether the observed systemic physiological influences of LNG-IUD are associated with mood disturbances or emotional lability. However, our results are in line with previous studies reporting an excess burden of affective symptoms in LNG-IUD users (18-20,40,41), and the recent findings of a large-scale longitudinal study suggesting a 34 percent higher risk of depression in women using the LNG-IUD (22).

In conclusion, our findings suggest that LNG-IUD robustly potentiates the systemic responses to psychosocial stress. Given the ACTH results demonstrating a centrally-mediated mechanism, it is likely that the levonorgestrel - IUD (Mirena®) is leaking a sufficient amount of progestin into the systemic circulation to sensitize hypothalamic/pituitary function which might influence mood and emotion.

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Chapter 6

Adrenocorticotropic hormone elicits gonadotropin secretion in premenopausal women

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Hum Reprod. 2016; 31(10):2360-2368

ABSTRACT

Study question: Does adrenocorticotropic hormone (ACTH) induce gonadotropin release in premenopausal women?

Summary answer: Administration of ACTH stimulates gonadotropin release, most likely by stimulation of the production of cortisol, in premenopausal women.

What is known already: In animal models, acute activation of the hypothalamic-pituitary-adrenal (HPA) axis has been shown to induce gonadotropin release in the presence of sufficiently high estrogen levels. However, it is unknown whether the HPA axis has a similar influence on gonadotropin release in humans.

Study design, size, duration: A mixed factorial design. A total of 60 healthy female participants participated in an experimental study.

Participants/materials, setting, methods: The study sample comprised three distinct hormonal-based populations: 1) lowPROG-lowE2, 2) lowPROG-highE2 and 3) highPROG-highE2 women. A low-dose (1 μg) of ACTH (Synacthen®) was administered to all study participants. Serum steroid and gonadotropin concentrations were measured prior to, and at 30 and 90 minutes after, intravenous ACTH administration.

Main results and the role of chance: Mean serum cortisol levels increased significantly following ACTH administration in all groups ($P<0.001$). Similarly, the serum levels of 17-OH-progesterone, androstenedione, dehydroepiandrosterone and testosterone increased significantly in all groups ($P < 0.01$). The lowPROG-highE2 and highPROG-highE2 groups exhibited a significant increase in LH and FSH levels ($P < 0.001$), whereas the lowPROG-lowE2 group demonstrated blunted LH and FSH responses to ACTH administration ($P < 0.05$).

Limitations, reasons for caution: Testing during the follicular phase of the natural menstrual cycle might have elicited premature, or more pronounced, LH surges in response to ACTH administration.

Wider implications of the findings: Our findings suggest a novel mechanism by which the adrenal cortex functions as a mediator of gonadotropin release. These findings contribute to a greater understanding of the influence of acute stress on reproductive endocrinology.

Study funding/competing interest(s): Erasmus University Medical Center

Trial registration number: EudraCT Number 2012-005640-14

INTRODUCTION

Psychosocial stress is a highly significant factor predicting health outcomes and quality of life (1). The best-studied physiological response to stress is mediated by the hypothalamic-pituitary-adrenal (HPA) axis, which can be affected by the hypothalamic-pituitary-gonadal (HPG) axis, regulating metabolism and reproductive function, respectively (2,3). Previous studies have also demonstrated that chronic persistent stress interferes with the release of hypothalamic gonadotropin releasing hormone (GnRH), resulting in a suppression of gonadotropin levels (4,5). Studies in animal models have elucidated candidate physiological mechanisms underlying the well-replicated finding of stress-induced reproductive suppression in humans (6–8). The female reproductive system is powerfully modulated by stress, often leading to chronic anovulation and amenorrhea during periods of persistent stress (9). In adolescents, chronic stress has been shown to significantly delay the onset of puberty (10).

Contrary to the effects of persistent stress, acute stress has been repeatedly shown to facilitate reproductive functioning by stimulating gonadotropin secretion (5,11,12). Animal studies have yielded a candidate hormonal mechanism through which acute stressors facilitate the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (13). Notably, the effect of acute stress on gonadotropin release is highly dependent upon the circulating level of estradiol (13–15). Moreover, adrenalectomy, but not ovariectomy, abolishes the facilitation of gonadotropin release by acute stress in rodents (15,16). Lastly, adrenal progesterone has been implicated as an important mediator of the stimulatory effect of stress on gonadotropins in the presence of an estrogen-primed environment (12,17). Taken together, widely convergent evidence in animal studies has given considerable support to the hypothesis that the facilitation of gonadotropin release by acute stress is mediated through adrenal steroids.

To date, studies regarding the effects of HPA-axis stimulation on LH release in humans have concluded that in postmenopausal women the LH response to adrenal stimulation is highly estrogen-dependent (15), and significantly potentiated by progesterone (18). However, it remains unknown whether gonadotropin release is facilitated by adrenal stimulation in premenopausal women, and the extent to which this may be governed by ovarian function.

Therefore, the aim of this study was to determine the influence of acute adrenocortical stimulation by administration of a low dose of ACTH on the release of LH and FSH in women with a normal menstrual cycle. In addition, we sought to explore the modulatory effect of estrogen and progestin on adrenal facilitation of gonadotropin release by administering a low dose of ACTH in three distinct healthy populations: 1) women having a natural menstrual cycle, 2) women taking oral contraceptive pills (a combination of estrogen/progestin), and 3) women using a progestin-releasing intra-

uterine device (IUD). Combination estrogen/progestin contraceptives have previously been shown to inhibit ovarian function (19,20). In comparison, the progestin-releasing IUD has been suggested to only partly and only during the first year inhibit ovarian function, leaving circulating estradiol within the normal range for women of reproductive age (21,22). Considering that LNG-IUD does not generally excrete sufficient amounts of progesterone to suppress the hypothalamic-ovarian axis (23), and given the possibility of a difference in gonadotropin release between young premenopausal women in the pre-ovulatory versus post-ovulatory phase, we reclassified the groups based on progesterone level and ovulatory phase. This study design provided us with the opportunity to compare the effects of acute stimulation of the adrenal cortex on gonadotropin release under conditions of intact ovarian function at different cycle phases, as well as in a setting of complete ovarian suppression**.** In addition, given previous studies reporting an association of hormonal contraceptives with emotional lability, anxiety, and depression (24), we performed structured assessments of the psychological affective state of our study participants in order to evaluate potential confounding effects.

SUBJECTS AND METHODS

Subjects

An *a priori* power analysis was performed at 80% power with a significance threshold of 0.05 in order to determine the cohort sample size. The power analysis indicated that a total sample size of 60 would provide confidence to detect differences of at least medium effect size between conditions. A total of 60 healthy women of reproductive age participated in this study (mean 22.83, SD 3.12, range 18-30 years). Subjects were recruited through local advertisements, and provided with monetary compensation (€50) for their participation. Hormonal contraceptive use was determined based on a structured questionnaire during the initial telephone screening, and reconfirmed on the day of testing. Women were considered eligible for the study only if they met one of the following inclusion criteria for continuous hormonal contraceptive use for at least the previous 4 months: 1) oral monophasic combined preparation containing ethinylestradiol (EE) 0.03mg and 0.15mg levonorgestrel (Ethinylestradiol/levonorgestrel, Microgynon® 30) [EE30/LNG group; N=20], 2) progestin-only LNG releasing IUD 0.02mg/24 hours (Mirena®; Bayer [LNG-IUD group; N=20], or 3) absence of any hormonal contraceptives and having a regular menstrual cycle length between 23 and 35 days (naturally cycling) [NC group; N=20]. The duration of LNG-IUD use ranged from 16 to 28 months. All participants had a normal menstrual cycle length between 26 and 29 days. Exclusion criteria were a history of clinically-significant psychiatric,

neurologic, endocrine or medical illness (including alcohol or drug dependence, asthma, allergies, cardiovascular disease, endometriosis, polycystic ovary disease, or gynaecologic infection), body-mass-index (BMI) <19 or >26 kg/m^2, atypical sleep pattern, the use of any prescription medication other than hormonal contraceptives within the previous 4 months, and pregnancy or lactation within the previous 12 months. Women in the EE30/LNG group were tested during the active pill weeks. NC women were tested in the luteal phase of the menstrual cycle, between days 20 and 27 of their cycle.

The study was conducted according to the declaration of Helsinki and was approved by the Medical Ethical Research Committee of the Erasmus MC, University Medical Center Rotterdam. All subjects provided written informed consent for their participation.

Psychological assessment

To examine the possibility that responses to ACTH administration could be confounded by differences in affect regulation between the contraceptive groups, all participants completed the Positive and Negative Affect Scale (PANAS), a well-validated questionnaire for measuring general, positive and negative affective states (Watson et al., 1988). Each of the 20 items is rated on a 5-point Likert scale ranging from 1 (very slightly or not at all) to 5 (extremely). The PANAS has been established to have high reliability (Positive affect scale: Cornbach's $\alpha = 0.89$, Negative affect scale: $\alpha = 0.85$) (25).

ACTH administration

Participants abstained from smoking, alcohol, caffeinated beverages, and physical exercise on the day of testing. There were no other dietary restrictions. Testing was conducted between 13.00 and 16.00h. The testing procedure began with a general medical examination to reconfirm the subject's physical and mental health status. An intravenous catheter was inserted either into the antecubital or the medial cubital vein to obtain serial blood samples. The intravenous catheter was flushed with normal saline immediately after each blood sampling time point. Following an initial 30 minute rest period, baseline venous blood samples were obtained for steroid and protein hormone assessments (cortisol, 17-hydroxyprogesterone [17-OH-progesterone], progesterone, testosterone, dehydroepiandrosterone [DHEA], androstenedione, dehydroepiandrosterone sulfate [DHEAS], and estradiol [E2]), globulin levels (corticosteroid binding globulin [CBG], sex hormone binding globulin [SHBG]), LH and FSH). Immediately following withdrawal of the baseline venous blood samples, a 1 µg IV bolus of 1-24 ACTH (Synacthen®; Novartis, Basel, Switzerland) was administered. Additional blood samples were obtained at 30 and 90 minutes following

ACTH administration. Subjects were asked to sit quietly in a semi-recumbent position throughout the entire procedure. No adverse events were reported.

Sample collection

Blood samples were collected using Vacutainer® tubes, immediately placed on ice upon collection and centrifuged at 4°C for 10 minutes at 3000 x g within 1 hour of collection. The resulting serum was aliquoted prior to storage at -80°C.

Hormone determinations

With the exception of estradiol, steroid hormones were measured using the LC-MS/MS method with the CHS™ MSMS Steroids Kit (Perkin Elmer, Turku, Finland). The Steroids Kit uses a combined solvent extraction and protein precipitation method with acetonitrile containing the deuterated internal standards $^{2}H_{5}$-androstenedione, $^{2}H_{3}$-cortisol , $^{2}H_{8}$-17-OH-progesterone, $^{2}H_{6}$-DHEA, $^{2}H_{9}$-progesterone, and $^{2}H_{5}$-testosterone. The internal standards undergo processing identical to the analytes. Chromatographic separation was performed on a Waters® (Milford, MA, USA) Acquity™ UPLC HSS T3 1.8 µm column (diameter 1 mm, length 10 cm) with acetonitrile/MeOH gradient, and in-line filters with 0.2 µm frits. A Waters® XEVO-TQ-S system equipped with an electrospray ionization (ESI) source operating in the electrospray positive mode was used except for DHEAS (negative ESI). Multiple reaction monitoring was applied for the detection of the analytes using both quantifiers and qualifiers.

The lower limits of quantification for androstenedione, cortisol, DHEA, DHEAS, progesterone, 17-OH-progesterone and testosterone were 0.20, 2.57, 2.2, 24.7, 0.13, 0.10, and 0.07 nmol/L, respectively. During the LC-step of the steroid assay, progesterone and 17-OHP were completely separated, thereby removing the possibility of cross-reactivity in this assay. Estradiol was measured by the Coat-A-Count radioimmunoassay of Siemens Healthcare Diagnostics Products (Los Angeles, CA, USA). Intra- and inter-assay coefficients of variation for the steroid assays were <7.0 and <8.0% for androstenedione, <6 and <6% for cortisol, <7 and <8% for DHEA, <8 and <13% for DHEAS, <6 and <7% for progesterone, <6 and <6% for 17-OH-progesterone, <6 and <9% for testosterone and <5 and <7% for estradiol. LH, FSH, and SHBG concentrations were measured using the Siemens Immulite XPi system. Serum CBG concentrations were determined by radioimmunoassay (DRG Instruments GmbH, Marburg, Germany). Intra- and inter-assay coefficients of variation were <4 and <7% for LH, <3 and 6% for FSH, <4 and <5% for SHBG and <9 and <11% for CBG.

Data analysis

Given the influence of menstrual phase (pre-ovulatory vs. post-ovulatory) on gonadotropin release, participants from the natural cycling and LNG-IUD groups were classified based on progesterone level. Women with progesterone concentrations above 5 nmol/l were classified in the highPROG/highE2 group (n=12) and women with a lower progesterone concentration in the lowPROG/highE2 group (n=28). Estradiol levels in these two groups were not different. Women using EE30/LNG were designated as lowPROG/lowE2 (n=20).

Statistical analyses were conducted using the SPSS statistical software package (IBM SPSS Statistics, Version 21). Results are expressed as means ± SEM, unless otherwise specified. Data per parameter were tested for normality of distribution and homogeneity of variance using Kolmogorov—Smirnov and Levene's tests. In six patients, one of the hormone measurements was not possible to quantify due to interfering peaks in the chromatogram (progesterone, n=1; E2, n=1; androstenedione, n=4). To meet the normality assumption, where necessary, hormonal data were logarithmically transformed. After log-transformation, the data were normally distributed. In order to examine group differences in demographic characteristics and affect, chi-squared tests and one-way ANOVAs were conducted. To analyse hormone profiles in response to ACTH administration, ANOVAs were performed with a repeated-measure factor Time (baseline, +30 minutes, +90 minutes), between-subject factor Group (lowPROG-lowE2, lowPROG-highE2, highPROG-highE2), and the interaction effect of Time x Group. Post hoc analyses, where necessary, were performed using Bonferroni multiple means comparisons. To reduce the possibility of a Type I error when analyzing steroids reactivity, statistical significance for these tests was defined at the more stringent threshold of $P < 0.01$. In order to check for potentially confounding effects of age, BMI, and PANAS scores on the steroid and gonadotropin responses, these parameters were first evaluated separately in a set of ANOVAs for repeated measures. Age, BMI, and PANAS scores did not yield significant main or interaction effects in relation to the steroid or gonadotropin responses. Therefore, these variables were not included as covariates in subsequent analyses.

Since ethinylestradiol influences levels of CBG, which binds cortisol with high affinity, CBG concentrations were included as covariates in analyses of cortisol concentrations. For general linear models (GLMs), F-values, degrees of freedom, and *P* values were corrected by the Greenhouse-Geisser procedure whenever the assumption of sphericity was violated. Effect sizes were calculated by partial eta squared (η^2). *P* values less than 0.01 were considered to be statistically significant.

RESULTS

Subject characteristics, and baseline ACTH and binding globulin levels

The groups did not differ significantly in age or BMI (Table 1). No significant differences were found in positive or negative affect scores on the PANAS, indicating comparable baseline affective states between the study groups (Table 1). The study groups were also similar in their baseline ACTH levels (Table 1). Importantly however, the lowPROG-lowE2 group exhibited significantly higher baseline CBG ($P < 0.001$) and SHBG levels ($P < 0.001$), due to the stimulating effect of the synthetic estrogen in the oral contraceptive (Table 1).

Effect of ACTH administration on gonadotropin release

ACTH administration resulted in significant time-dependent changes of LH and FSH levels in all groups (lowPROG-highE2 and highPROG-highE2, $P < 0.001$; lowPROG-lowE2, $P < 0.05$). The groups differed significantly regarding LH levels, with the lowPROG-lowE2 group displaying overall lower LH concentrations ($P < 0.001$; post hoc: lowPROG-highE2 = highPROG-highE2 > lowPROG-lowE2; Figure 1a). No significant Group x Time interaction effect was observed. The FSH levels differed significantly between the study groups ($P < 0.001$). A significant Group x Time interaction was observed ($P < 0.05$; post hoc: lowPROG-highE2 > highPROG-highE2 > lowPROG-lowE2; Figure 1b): the EE30/LNG group displayed a blunted FSH response to ACTH administration ($P < 0.01$).

Table 1. Subject characteristics, affect state, and baseline globulin and ACTH levels of the experimental groups

	lowPROG/ highE2	**highPROG/ highE2**	**lowPROG/ lowE2**
	(n=28)	*(n=12)*	*(n=20)*
Age, mean (SD), years	23.04 (3.26)	23.42 (4.64)	22.2 (1.47)
BMI, mean (SD), kg/m^2	22.16 (2.11)	21.87 (1.30)	22.53 (2.89)
PANAS			
Positive Affect scale, score	28.14 (5.82)	28.58 (3.87)	29.45 (6.89)
Negative Affect scale, score	13.75 (3.23)	13.17 (2.73)	12.00 (2.15)
SHBG, mean (SD), µg/ml	25.76 (8.53)	26.41 (8.94)	50.94 (14.69)*
ACTH, mean (SD), µg/ml	3.40 (1.37)	2.03 (1.07)	3.07 (3.35)
CBG, mean (SD), µg/ml	52.91 (8.60)	57.08 (6.11)	120.99 (22.11)*

Abbreviations: Positive affect and Negative affect scale (PANAS), cortisosteroid binding globulin (CBG), sex hormone binding globulin (SHBG), adrenocorticotropic hormone (ACTH).
*One-way ANOVA between three experimental groups, $P < 0.001$.

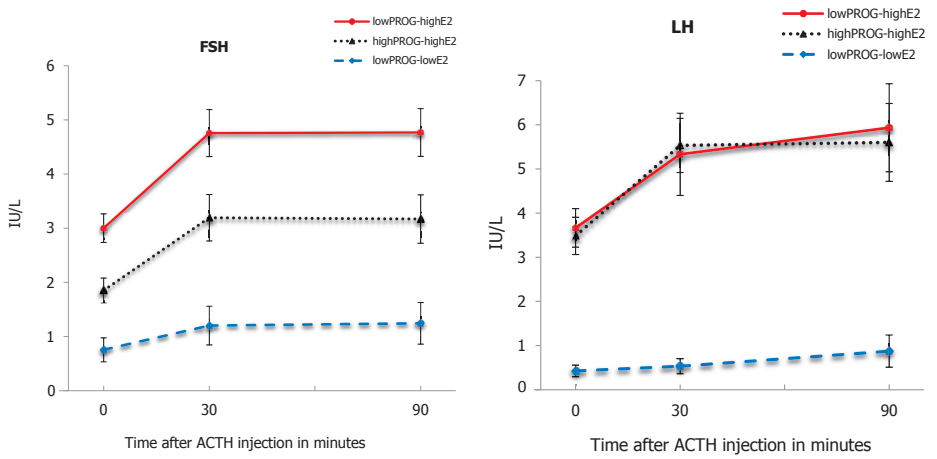

Figure 1. Untransformed raw LH (**a**) and FSH (**b**) mean ± SEM values at baseline, +30, and +90 minutes after administration of low dose (1µg) ACTH.

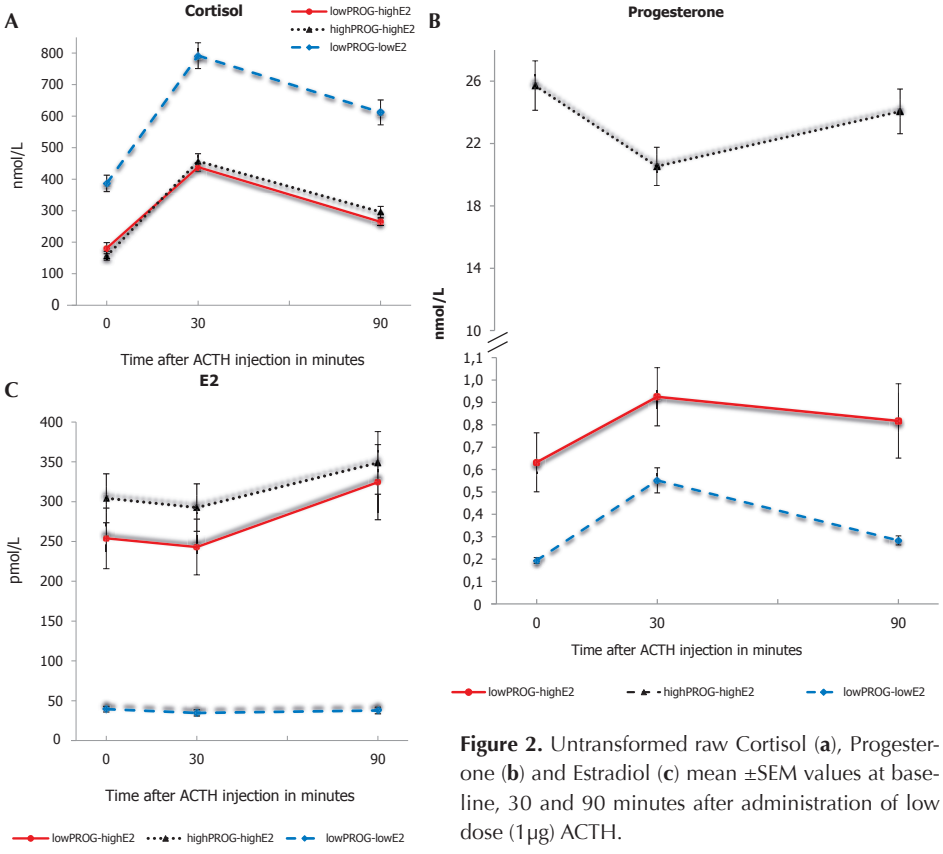

Figure 2. Untransformed raw Cortisol (**a**), Progesterone (**b**) and Estradiol (**c**) mean ±SEM values at baseline, 30 and 90 minutes after administration of low dose (1µg) ACTH.

Effects of ACTH administration on the steroid profile

ACTH administration resulted in significant time-dependent changes in the levels of cortisol, 17-OH-progesterone, progesterone, testosterone, DHEA, DHEAS, and androstenedione ($P< 0.001$ for each group x steroid combination), all displaying significant increases at 30 minutes after ACTH administration ($P < 0.01$ for each group x steroid combination). With regard to E2, a significant increase was observed 90 minutes after ACTH administration in the lowPROG-highE2 and highPROG-highE2 groups ($P< 0.001$ for each group), but no change was found in the lowPROG-lowE2 group.

Cortisol. The study groups differed significantly with regard to total serum cortisol levels. Women using oral contraceptives (lowPROG-lowE2 group) exhibited significantly higher mean total cortisol levels, compared to the lowPROG-highE2 and highPROG-highE2 groups ($P< 0.001$; Figure 2a). However, after controlling for CBG levels, no significant group or interaction effect remained, confirming the influence of CBG on cortisol levels.

Progesterone. The study groups differed significantly regarding progesterone levels, with the highPROG-highE2 group showing higher overall progesterone than the lowPROG-highE2 and lowPROG-lowE2 groups ($P < 0.001$). ACTH administration induced a significant increase in progesterone in the lowPROG-highE2 and lowPROG-lowE2 groups, but not in the highPROG-highE2 group ($P < 0.001$; Figure 2b).

17-OH-progesterone. 17-OH-progesterone levels differed significantly between the study groups at baseline, +30 and +90 minutes post-ACTH administration ($P < 0.001$; post hoc: highPROG-highE2 > lowPROG-highE2 > lowPROG-lowE2). Furthermore, a significant Group x Time interaction effect was observed ($P < 0.001$), with the lowPROG-lowE2 group displaying relatively higher 17-OH-progesterone increases at 30 minutes post-ACTH administration compared to the highPROG-highE2 and lowPROG-highE2groups (Table 2).

Androstenedione. Androstenedione levels differed significantly between the groups ($P < 0.001$; post hoc: highPROG-highE2 = lowPROG-highE2 > lowPROG-lowE2), with the lowPROG-lowE2 group displaying overall lower androstenedione levels, compared to the highPROG-highE2 and lowPROG-highE2 groups (Table 2). No significant Group x Time interaction effect was observed.

Dehydroepiandrosterone. The study groups differed significantly in DHEA concentrations at baseline, and +30 and +90 minutes post-ACTH administration ($P < 0.001$; post hoc: highPROG-highE2 = lowPROG-highE2 > lowPROG-lowE2; Table 2). No significant Group x Time interaction effect was observed.

Dehydroepiandrosterone sulfate. DHEAS levels differed significantly between the study groups ($P < 0.01$; post hoc: highPROG-highE2 = lowPROG-highE2 > lowPROG-lowE2), with the highPROG-highE2 and lowPROG-highE2 groups displaying higher

Table 2. Adrenal steroid levels in response to ACTH stimulation in the experimental groups

		lowPROG/ highE2	**highPROG/ highE2**	**lowPROG/ lowE2**	P-value within group	P-value Between group
Cortisol (nmol/L)		*(n=28)*	*(n=12)*	*(n=20)*		
	Baseline	182.58 (89.16)	158.82 (41.55)	386.60 (116.99)		
	30 min	435.73 (61.28)	456.86 (77.89)	791.81 (183.24)	P < 0.001	P < 0.001
	90 min	261.26 (56.25)	295.48 (65.88)	612.08 (176.64)		
Progesterone (nmol/L)		*(n=28)*	*(n=11)*	*(n=20)*		
	Baseline	0.63 (0.50)	25.71 (16.88)	0.19 (0.06)		
	30 min	0.93 (0.68)	20.53 (12.77)	0.55 (0.25)	P < 0.001	P < 0.001
	90 min	0.81 (0.78)	24.06 (14.45)	0.28 (0.09)		
17-OH Progesterone (nmol/L)		*(n=28)*	*(n=12)*	*(n=20)*		
	Baseline	1.25 (0.76)	4.35 (1.89)	0.19 (0.13)		
	30 min	2.39 (0.98)	5.79 (2.69)	1.49 (0.71)	P < 0.001	P < 0.001
	90 min	1.41 (0.69)	4.38 (1.90)	0.39 (0.21)		
Androstenedione (nmol/L)		*(n=27)*	*(n=12)*	*(n=17)*		
	Baseline	4.01 (1.70)	3.90 (1.48)	1.73 (.71)		
	30 min	5.48 (2.27)	5.51 1.33)	2.61 (0.84)	P < 0.001	P < 0.001
	90 min	4.16 (1.61)	4.01 (1.20)	1.92 (0.63)		
DHEA (nmol/L)		*(n=28)*	*(n=12)*	*(n=20)*		
	Baseline	21.50 (10.43)	16.84 (5.48)	11.18 (4.91)		
	30 min	43.73 (15.95)	45.31 (12.90)	24.52 (10.73)	P < 0.001	P < 0.001
	90 min	21.25 (8.57)	20.52 (7.48)	11.85 (3.87)		
DHEAS (µmol/L)		*(n=28)*	*(n=12)*	*(n=20)*		
	Baseline	5.15 (2.43)	6.06 (2.73)	4.10 (1.69)		
	30 min	5.34 (2.53)	5.87 (2.16)	4.21 (1.68)	P < 0.01	P = 0.02
	90 min	5.15 (2.29)	5.97 (2.31)	4.06 (1.66)		
Testosterone (nmol/L)		*(n=28)*	*(n=12)*	*(n=20)*		
	Baseline	0.97 (0.36)	1.04 (0.45)	0.55 (0.18)		
	30 min	1.11 (0.36)	1.13 (0.36)	0.70 (0.23)	P < 0.001	P < 0.001
	90 min	1.08 (0.40)	1.10 (0.41)	0.58 (0.17)		
E2 (pmol/L)		*(n=27)*	*(n=12)*	*(n=20)*		
	Baseline	253.96 (197.80)	304.27 (106.38)	39.44 (15.97)		
	30 min	243.00 (181.95)	292.58 (103.17)	34.71 (17.47)	P < 0.001	P < 0.001
	90 min	324.45 (244.79)	348.72 (136.16)	37.80 (18.49)		

Data are presented as mean ± SD.

overall levels when compared to the lowPROG-lowE2 group (Table 2). No significant Group x Time interaction effect was observed.

Testosterone. The lowPROG-lowE2 group exhibited overall lower testosterone levels, compared to the highPROG-highE2 and lowPROG-highE2 groups ($P < 0.001$; Table 2). A significant Group x Time interaction effect demonstrated a larger increase of testosterone levels following ACTH administration in the lowPROG-lowE2 group, compared to the highPROG-highE2 and lowPROG-highE2 groups ($P < 0.01$).

Estradiol. E2 levels were significantly different between the study groups: the lowPROG-lowE2 group had lower E2 levels than the highPROG-highE2 and low-PROG-highE2 groups ($P < 0.001$). No differences were observed in E2 levels between the highPROG-highE2 and lowPROG-highE2 groups. ACTH administration induced a significant increase of E2 in the highPROG-highE2 and lowPROG-highE2 groups, but not in lowPROG-lowE2 users ($P < 0.001$; post hoc: NC = LNG-IUD > EE30/LNG; Figure 2c).

DISCUSSION

The aim of our study was to examine the influence of acute adrenal cortex stimulation on gonadotropin release in 3 groups of premenopausal women distinguished by the different levels of progesterone and estradiol: highPROG-highE2, lowPROG-highE2 and lowPROG-lowE2. Basal hormone levels differed between groups on the basis of cycle phase (progesterone and 17-OH progesterone in the highPROG-highE2 and lowPROG-highE2 groups), and on the basis of suppression of LH and FSH in the female group using oral contraceptives, lowPROG-lowE2 leading to suppression of the ovarian component of the production of androgens and estradiol.

Steroid-dependent regulation of gonadotropin release has been shown to involve a complex interaction with estrogen, as observed in studies of estrogen-replacement therapy in postmenopausal women, in which activation of the HPA axis resulted in gonadotropin release only in the presence of sufficient levels of circulating estrogen (15). In our study, estrogens were present in all study groups: endogenous estradiol in the highPROG-highE2 and lowPROG-highE2 groups, and ethinylestradiol in the lowPROG-lowE2 group. Further evidence that adrenal steroid secretion is associated with gonadotropin release comes from animal studies in which both adrenalectomy and pre-treatment with RU486, which has antiglucocorticoid and antiprogesterone activities, each abolished stress-induced gonadotropin release (12,17,26). Similar to the results of human studies, the stimulatory effect of ACTH was observed only in estrogen-primed rats, consistent with the essential requirement of adequate estradiol (12,16).

In our data, a significant increase of ACTH-induced gonadotropin levels was observed in all groups. Among women with low levels of progesterone, ACTH administration led to increased progesterone in the presence of normal estradiol levels. This permissive hormonal context is comparable to that in the beginning of the midcycle peak of LH and FSH (27). Earlier research has established that estradiol and progesterone influence the induction of the midcycle gonadotropin surge (28,29). In our study, adrenal stimulation by ACTH caused a near doubling of the relatively low progesterone levels in the lowPROG-highE2and lowPROG-lowE2groups. However, in the highPROG-highE2 group, in which estradiol levels were comparable to those in lowPROG-highE2 group, a similar increase in gonadotropin levels was observed in the absence of increased progesterone levels. Therefore, the analogous ACTH effects on gonadotropin release in the highPROG-highE2 and lowPROG-highE2 groups suggests that progesterone is unlikely to be mediating the increase in LH and FSH.

Alternative mechanisms to explain the ACTH-induced release of LH and FSH might involve the influence of 17-OH-progesterone, androgens, estradiol or cortisol. In our study, the relative effect of ACTH on circulating levels of 17-OH-progesterone was even larger than observed for progesterone, in accordance with previous studies (30). Elevated levels of 17-OH-progesterone are in line with earlier reports showing that peripheral levels of 17-OH-progesterone during the luteal phase of the cycle are higher than those during the follicular phase (31). It has recently been described that 17-OH-progesterone may have glucocorticoid activity due to its binding to the glucocorticoid receptor (GR) and its ability to transactivate the GR *in vitro* (32). However, considering that 17-OH-progesterone binds weakly to the GR and is a less potent agonist of GR than cortisol, it is unlikely that the observed gonadotropin increase in our study is mediated by 17-OH-progesterone (32). Furthermore, although earlier research in rhesus monkeys has suggested that 17-OH-progesterone may facilitate the onset of LH surges (33), the stimulating effect of 17-OH-progesterone on LH release was not found in humans (34). This makes it unlikely that the increase of 17-OH-progesterone caused the surge of gonadotropins.

Regarding the influence of increased levels of androgens and estradiol in the ACTH-induced release of gonadotropins, it is very unlikely that these steroids function prominently, since only suppressive effects of androgens have been described in patients with androgen producing tumours (35,36), or in rats (37). Moreover, the increase of estradiol levels was detectable only 90 minutes after the administration of ACTH, whereas the surge of LH and FSH was already evident after 30 minutes.

Taken together, we believe that cortisol is the most parsimonious mediator of the increased levels of LH and FSH after ACTH injection. This is in accordance with the results of experiments in rats, in which glucocorticoids have been shown to affect gonadotropin release via receptor mediated mechanisms (38), and for which GR

activity has been shown to modulate LH through both pituitary and neuroendocrine mechanisms following exposure to stress (39–41).

The present study has several limitations. Because this is a secondary data analysis, examining the impact of acute adrenal stimulation on gonadotropin release was not the primary goal when designing the original study. Therefore, women having a natural menstrual cycle were tested during their luteal phase. Testing during the follicular phase of the menstrual cycle might have elicited premature, or more pronounced, LH surges in response to ACTH administration. However, reclassification of our data based on different progesterone levels though similar estradiol concentrations did not change the findings. Additionally, women were not randomly assigned to the study, but were recruited based on their use of contraceptives. However, the groups were very similar for all known confounding variables, including general medical health, age, BMI, affective state.

While it is likely that the increase of gonadotropins observed in this study are due to a mediating effect of cortisol, it is also possible that administration of the ACTH might have resulted in downstream adaptations to CRH through a secondary feedback loop. However, the low-dose (1µg) ACTH stimulation test has been well documented to be more physiological and sensitive than for example the higher-dose (250µg or 100µg) ACTH stimulation tests. The 1µg low-dose administration results in a maximal serum ACTH concentration of 200 ng/l, which is of a similar order of magnitude as observed in venous blood samples from the sinus petrosus inferior (W.W. de Herder, unpublished data). Therefore, it seems unlikely that a 1µg dose of ACTH directly affects pituitary function, in addition since the extensive literature of investigations using the same low-dose ACTH formulation has never previously reported direct alteration of pituitary function. Furthermore, we acknowledge the lack of prolactin measurements which might have provided better insight into the stress induced gonadotropin release. However, considering that prolactin is released from the anterior pituitary and our focus was on the effects of adrenal stimulation, we considered the effect of prolactin to be negligible.

In conclusion, our data are the first to demonstrate that acute stimulation of adrenal steroid production, most likely cortisol, mediates enhanced gonadotropin release in healthy premenopausal women. More generally, these findings contribute to an improved understanding of the influence of acute stress on reproductive endocrinology.

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Chapter 7

General Discussion

MAIN FINDINGS

Psychosocial stress is an inevitable component of our daily lives, which can affect both our mental and physical health. Therefore, detailed knowledge of the psychobiological pathways linking stress and psychopathology is of major importance. The studies presented in this thesis report data on stress regulation in women with and without personality disorders. The aims of the studies were: a) to explore the fundamental personality characteristics that contribute to cognitive appraisals of psychosocial stress, b) to expand our knowledge of the underlying biological mechanisms of stress responses in women with and without personality disorder, and c) to shed light on the factors that modulate physiological reactivity to acute psychosocial stress. For these purposes, healthy women and women diagnosed with a personality disorder (Borderline personality disorder and Cluster C personality disorder) were administered the Trier Social Stress Test (TSST). Cognitive appraisals, as well as psychological and physiological responses, were assessed before, during and after the TSST. In addition, to improve our understanding of the effects of sex hormones on stress induced physiological responses, we administered low-dose (1µg) intravenous adrenocorticotropic hormone (ACTH) in an additional sample of healthy women.

The main findings of our studies demonstrated that (see Figure 2 for schematic representation):

- when exposed to a challenging situation, cognitive perception of stress was augmented directly by emotional dysregulation, and indirectly by attachment style and temperament. Positive affectivity contributes to preservation against stress, which might be seen as a key to resilience. Notably, all of the observed associations between attachment styles, temperament and cognitive stress appraisals, including the mediating role of maladaptive personality traits, applied independently to women with low and high burden of psychopathology. These findings underline the importance of dysfunctional traits in understanding the role of individual characteristics on cognitive appraisals of acute psychosocial stress.
- the 5-HTTLPR genotype was found to be associated with cortisol responsivity to psychosocial stress. Women with the LL genotype demonstrated significantly higher salivary cortisol responses to psychosocial stress than women with at least one copy of the S allele. Additionally, our results showed that early life adversities did not modulate the effects of the *SLC6A4* 5-HTTLPR genotype on salivary cortisol responses to psychosocial stress in women.
- women suffering from either BPD or CPD exhibited similarly robust mood disturbances in response to acute psychosocial stress. However, patients with BPD demonstrated significant attenuations of salivary cortisol levels and heart rate reactivity as compared to patients with CPD or healthy controls. Thus, this pattern

of blunted cortisol and heart rate reactivity to psychosocial stress appeared to be specific for patients with BPD.

- distinct hormonal contraceptive methods have contrasting effects on endocrine reactivity to psychosocial stress. Compared to naturally cycling women, women using an LNG-IUD exhibited a robust potentiation of their salivary cortisol response during the TSST. However, women using combination estrogen-progestin contraception showed a relatively blunted cortisol response versus naturally cycling women. Moreover, women using an LNG-IUD had a greater potentiation of heart rate responsivity to the TSST than women using oral combination estrogen-progestin contraception. In line with these findings, women using an LNG-IUD exhibited significantly higher concentrations of hair cortisol than naturally cycling women.
- acute stimulation of adrenal steroid production, by means of a low-dose ACTH challenge test, mediated enhanced LH and FSH release in healthy premenopausal women. In addition, our findings confirmed a permissive function of estradiol, i.e. activation of the HPA axis, resulting in gonadotropin release only in the presence of sufficient levels of circulating estrogen.

COGNITIVE APPRAISALS

Given the central role of cognitive appraisal in the process of stress regulation and its potential to mediate endocrine responses to environmental demands, we explored how fundamental personality characteristics, attachment and temperament modulated cognitive appraisals of acute stress. Furthermore, we examined whether the potential relationships between personality characteristics and cognitive appraisals were mediated by emotional dysregulation. Cognitive appraisals were assessed during the anticipation period of a pending acute psychosocial stressor.

The findings of this thesis imply that, when exposed to a challenging situation, individuals with high positive affectivity judged themselves as being more capable and having sufficient coping resources to confront stressful situations. We did not observe direct associations between attachment insecurities and cognitive stress appraisals. However, our model showed that the significance of cognitive perception can be augmented by emotional dysregulation. This finding provides additional evidence that maladaptive personality traits are important factors in understanding the contribution of individual characteristics to cognitive appraisals of acute psychosocial stress. Remarkably, the analyses revealed that the same pathway, with a mediating role of dysfunctional traits, applied to both healthy controls and women with personality disorders. From a clinical perspective, this finding supports the generally

accepted approach that all people have a mix of personality traits that are adaptive and functional, and traits that are less optimal and might lead to increased stress susceptibility (1,2). Consequently, this might influence how individuals dynamically adapt to environmental demands, resulting in the subjective experience and objective physiological state of well-being.

GENETIC FACTORS

In order to further assess the factors that enhance stress vulnerability, we aimed to shed light on the role of specific genetic factors on the variability of the cortisol response to acute psychosocial stress. Gene variants of the serotonin transporter have been associated with vulnerability to stress-related disorders and HPA-axis reactivity to stress (3–5). The findings reported in *Chapter* 3 support the theory that functional genetic variation is associated with cortisol responsivity to psychosocial stress. However, whereas earlier studies have found that particularly homozygous S allele carriers are associated with an augmented cortisol response to a stress test (6,7), we found the opposite association, i.e. women with the LL genotype demonstrated significantly higher salivary cortisol responses to psychosocial stress than women with at least one copy of the S allele. These opposing findings could be partly due to differences in age and hormonal status. The majority of earlier studies on 5-HTTLPR and salivary cortisol reactivity were performed using young subjects, including newborns and adolescents (6,8,9), whereas our study included older women, most likely with a different hormonal status than adolescent girls. Apart from the well-known effects of the menstrual cycle on the HPA axis, several studies suggest that ovarian steroids (estradiol and progesterone) have a strong influence on serotonin synthesis, and expression of serotonergic receptors and 5-HTT (10–12), indicating that the effects of 5-HTTLPR on brain activity in women may change with alterations in hormonal status. Our findings indicate the need to further clarify the sex-specific biological interaction between the serotonergic system and ovarian hormones. These important factors are unfortunately often overlooked in studies combining data from male and female subjects.

We were not able to confirm a moderating effect of early life adversities on the 5-HTTLPR effects on cortisol response to stress. Our results show that childhood (the first 15 years of life) maltreatment is unlikely to account for the modulating role of the 5-HTTLPR genotype in women. However, different measures of adversity or less accurate classification and timing of these adversities might have been the reason of the divergent findings. Future studies are needed to further explore the interaction of 5-HTTLPR and environmental adversity on cortisol responses to stress, through

increasingly precise definitions of adverse life events and more detailed biomarker analyses including genome-wide DNA methylation profiling, which has recently been shown to be informative (13).

PSYCHOPATHOLOGY

There is ample evidence that patients with personality psychopathology experience an elevated perception of threat and have difficulties regulating their affect. However, the evidence for biological sensitivity is more ambiguous (14). In order to improve our understanding of the underlying biological mechanisms of emotional dysregulation in women with personality disorders, we conducted a study comparing emotional and physiological responses to psychosocial stress across three groups: outpatients with cluster C personality disorder (CPD), outpatients with borderline personality disorder (BPD), and healthy controls.

In response to the TSST, patients with CPD and BPD reported significantly higher subjective mood disturbance compared to healthy controls. Despite their similar subjective experience, BPD patients showed a distinct pattern of cortisol levels: significantly reduced cortisol levels at baseline and a blunted response to the TSST. In contrast, CPD patients tended to have heightened cortisol levels, both at baseline and in stress induced responses. Furthermore, BPD patients demonstrated a blunted heart rate response to the TSST, whereas CPD patients and healthy controls had nearly identical heart rate responses. In contrast to the attenuated pattern of heart rate reactivity, the BPD group exhibited a significantly higher overall SCL. SCL was similar between CPD patients and healthy controls. Additional analyses suggested that these results could not be explained by the presence of comorbid psychopathology such as post-traumatic stress disorder or eating disorders. Furthermore, in line with our expectations, we found that participants with higher levels of childhood trauma and/or increased attachment related anxiety exhibited attenuated cortisol and heart rate responses to the TSST, analogous to the patient group with BPD. This is not surprising, as it is well know that individuals who experience childhood trauma and related factors leading to insecure attachment, are heavily overrepresented among BPD patients. Moreover, it should be noted that a complex interaction of causal factors and comorbidities is present in patients with personality disorders. Patients with BPD and CPD are often burdened with co-morbid psychiatric illnesses, such as eating disorders and/or post-traumatic stress disorder (15–17). Nevertheless, we found that patients with BPD, in contrast to patients with CPD, manifest a distinct psychophysiological responsivity to psychosocial stress, indicating a potentially distinct underlying biology.

It should be mentioned that this study was based on a cross-sectional design, which precluded firm conclusions regarding the causality of the observed results. Furthermore, we acknowledge that we were not able to perform a semi-structured interview for Axis II diagnoses in order to make a comprehensive assessment of the patients included in this study. Hence, we might have missed some comorbid diagnoses, which therefore cannot be completely ruled out as a potential confounder of our findings. However, patients were clinically referred and the diagnoses were made by qualified and experienced psychotherapists, based on the DSM-IV criteria for personality disorders. Furthermore, we relied on self-report data of early childhood trauma, which is sensitive to uncertainty regarding the extent to which retrospective reports of early life adversities reflect the actual behavior of caregivers versus the subjective experience of them. However, current studies show that retrospective reports are well correlated with prospectively collected data (18). Above all, depending on the cohort and evaluation method, up to 81% of people with BPD report a history of childhood trauma (19), which is consistent with the widely held view that a lack of secure attachment is essential to the development of borderline psychopathology. Therefore, it is unlikely that retrospective accounts have significantly influenced the findings. However, future studies are required in larger cohorts to better identify risk and resilience factors that regulate autonomic and HPA axis dysfunction in BPD versus CPD.

EXOGENOUS HORMONAL FACTORS

In western countries, nearly half of all women of reproductive age rely on some method of hormonal contraception. Yet we know surprisingly little about how these exogenous hormones influence stress reactivity. Part of our study examined the effects of hormonal contraception on female stress induced physiology (*Chapter* 5). We focused on combination oral contraceptive pills and the levonorgestrel-releasing intrauterine device (LNG-IUD). During the last few years, women have been increasingly opting for the LNG-IUD given its widespread clinical endorsement as a safe, reliable method with negligible systemic effects (20). However, the findings reported in *Chapter* 5 do not support these claims. Relative to naturally cycling women, women using an LNG-IUD exhibited a robust potentiation of the salivary cortisol response to the TSST, whereas women using combination estrogen-progestin contraception exhibited a blunted cortisol response. Moreover, women using an LNG-IUD experienced a greater than 10 beats/min potentiation of their heart rate responsivity to the TSST. To confirm the hypothesis of the systemic effects of the LNG-IUD on stress induced physiology, we directly stimulated the adrenal cortex by administering a low-dose ACTH. In women using combination estrogen-progestin

contraception, this stimulation resulted in a blunted salivary cortisol response, analogous to the outcome following the TSST. Notably, this finding is consistent with the well-established estradiol-induced increase in cortisol binding globulin (CBG) levels, thereby enhancing the buffering capacity of serum cortisol with a reduction of the unbound fraction (21,22). In contrast, the blunted salivary cortisol response to ACTH in women using an LNG-IUD, which led to a potentiated cortisol response during the TSST, occurred despite the presence of a normal CBG level. Together, these findings suggest a homeostatic downregulation of adrenal cortex function in LNG-IUD users secondary to the chronic potentiation of acute cortisol responsivity. The LNG-IUD appears to induce both a centrally-mediated potentiation of HPA reactivity and a peripheral downregulation of adrenal cortex reactivity. Investigation of hair cortisol concentrations, reflective of chronic naturalistic cortisol secretion, demonstrated that, similar to the findings of the TSST, women using an LNG-IUD had significantly higher concentrations of hair cortisol than naturally cycling women. Conversely, women using combination estrogen-progestin contraception had significantly decreased hair cortisol levels. Therefore, our findings confirm the systemic influence of LNG-IUD on HPA axis functioning under both acute stress, as well as through daily life stress.

STRESS AND THE REPRODUCTION SYSTEM

Systems activated by stress can influence reproduction at the hypothalamic, pituitary and gonadal levels (23). It has been well recognized that reproductive function is suppressed under stressful conditions (24). However, stress has been demonstrated to exert both inhibitory and stimulatory effects on reproductive function, dependent on the length of stress exposure and the background of estrogen priming (24). Previous studies using animal models have suggested that acute stress leads to facilitation of gonadotropin release through stimulation of the HPA axis. However, despite increasing scientific attention to the deleterious impact of stress on reproductive health, no previous studies have ever examined whether gonadotropin release is influenced by acute HPA axis stimulation in premenopausal women.

In *Chapter* 6, we reported that acute administration of ACTH significantly enhances gonadotropin release in healthy premenopausal women. We examined this effect in 3 independent groups defined by their differential use of hormonal contraceptives: 1) women having a natural menstrual cycle, 2) women using oral contraceptives (combination estrogen/progestin), and 3) women using a levonorgestrel-releasing intrauterine device (IUD). With this study design, we have been able to further dissect the hormonal context by which adrenal cortex activity mediates gonadotropin release. Notably, our results confirm a permissive function of estradiol and sug-

gest a novel mechanism by which cortisol functions as an important mediator of gonadotropin release. Our data suggested that acute stimulation of adrenal steroid production, most likely cortisol, mediates enhanced gonadotropin release in healthy premenopausal women.

The interpretation of these findings might be limited by the cycle phase during testing. While women having a natural menstrual cycle were tested during their luteal phase, testing during the follicular phase of the menstrual cycle might have elicited premature, or more pronounced, LH surges in response to ACTH administration. Additionally, women were not randomly assigned to the study, but were recruited based on their use of contraceptives. However, the groups were very similar across many potentially confounding variables, including general medical health, age, body-mass index (BMI), and affective state. Although it is likely that the increase of gonadotropins observed in this study are due to a mediating effect of cortisol, it is also possible that administration of ACTH might have resulted in downstream adaptations to CRH through a secondary feedback loop. However, the low-dose (1µg) ACTH stimulation test has been well documented to be more physiological and sensitive than higher-dose (250µg) ACTH stimulation. Therefore, it seems unlikely that a 1µg dose of ACTH directly affects pituitary function. Future studies with different pharmacological challenge tests, such as Dex/CRH administrations, are needed to expand our knowledge of HPA axis physiology and in particular, female reproductive functioning.

Strengths and limitations

The strengths of the presented studies include the use of a realistic and standardized social stress procedure, the inclusion of clinically referred patient samples, the use of well-defined contraceptive use groups and the measurement of multiple response systems (i.e., HPA axis, ANS, and subjective emotional experiences) through the full trajectory of the stress response, from baseline to recovery. In addition, we made thorough efforts to control for potential sources of bias. Moreover, in order to hold the known influential factors constant across subjects, all participants were enrolled and examined under strictly standardized conditions, including matching for age, BMI, medication, hormonal contraceptives, and time of day and menstrual cycle phase during testing. All measurements were performed in the afternoon, between 14.00 and 16.00 hours to minimize circadian influences on salivary and physiological assessments. Participants were asked to abstain from caffeine and intense physical activity for at least 24 hours prior to the session, and to have been awake for at least 5 hours prior. Women having a natural cycle were tested during the luteal phase of the menstrual cycle.

In addition, all participants were carefully evaluated using a general health assessment, comprehensive self-report questionnaires and a structured clinical interview

for Axis I disorders. All of the included patients were diagnosed with personality disorder and were receiving psychotherapy at a mental health clinic. Healthy controls were a representative sample of women of reproductive age, and recruited from the general community. Assessment of attachment and childhood trauma allowed for the examination of these factors as potential influences on emotional reactivity in women with and without personality disorder.

The original version of the TSST, i.e. mock job interview plus mental arithmetic tasks in front of a real panel of judges, was highly effective in eliciting a stress response in every participant. Social evaluative threat and uncontrollability have been shown to be the major characteristics of the TSST explaining its effectiveness (25). Subjective reporting of disturbed mood in response to the TSST procedure suggested that all participants became personally involved in the task and found it to be highly stressful and disturbing. Notably, the majority of patients experienced the TSST as an overwhelming procedure which quite often resulted in outbreaks of emotional reactions such as anger, crying, sorrow or aggressive behavior. However, patients were able to complete the testing despite severe emotional reactions. One might argue whether a laboratory stressor consisting of these two essential elements, social evaluation and uncontrollability, is a proper reflection of the actual distress that patients face in their daily lives. Most of the time, they can use more avoidant stress regulation strategies to control the situation they face. On the other hand, in many naturalistic situations social evaluative threat and uncontrollability are strongly interconnected. For example, the behavior of the interaction partner in many situations cannot always be predicted. Also, in order to study HPA axis reactivity properly, a robust stressor is needed to evoke reliably significant elevations of free unbound cortisol.

Finally, the use of noninvasive sampling methods has both advantages and disadvantages. A noninvasive procedure improves patient recruitment. For example, saliva sample collection compared with blood sample collection increases patient acceptability and compliance (26). In addition, saliva sampling comes at a lower cost and allows an accurate determination of free unbound cortisol, which is, in contrast to protein-bound cortisol, responsible for cortisol's hormonal physiological function. However, saliva testing restricted us from further investigation of other important stress related hormones and globulins, for which blood samples are essential. Given that the TSST requires central processing, the assessments of cortisol releasing hormone and adrenocorticotropin hormone, together with cortisol, might have allowed the determination of more characteristic stress response patterns between the groups at different HPA axis levels. A similar need for blood sampling applies for the examination of genetic factors. However, saliva samples were viable alternatives for DNA extraction to perform genotyping of the serotonin transporter polymorphism. When studying the impact of contraceptives on the cortisol response following psy-

chosocial stress, the assessment of globulins that could have been studied in blood samples, which might function as important regulators of HPA axis responses, would have been useful. However, in the setting where we performed our TSST study, blood sampling was not a feasible option. Nevertheless, we were able to address some of these issues in our additional study, in which we administered a low-dose of ACTH to stimulate the adrenal cortex in healthy women. Future studies assessing a broader profile of steroid responses are needed in patients with personality disorders as well, to achieve a better understanding of the underlying biology of the disorders.

FUTURE PERSPECTIVES

The findings of this thesis underline the need to improve our understanding of the factors that increase vulnerability to stress. When considering normal homeostatic responses to environmental stressors, future research should aim to study both sexes, and take into account the hormonal status of the participants, especially in women. Fortunately, the inclusion of women in research trials and experimental designs is becoming increasingly more common. Although there is a general acceptance that inclusion of women in research studies is necessary for valid inferences about health and disease in women, stress research focused on women is growing less rapidly compared to men. Plausible reasons for this is the importance of considering women's reproductive status when assessing variations in HPA axis functioning and physiological stress levels. Due to hormonal fluctuations across the menstrual cycle, research in women is considered more costly and time consuming, and therefore quite often accompanied with high drop-out rates due to inaccurate self-reports of menstrual cycle phase (27). In addition, the recruitment of women who do not use hormonal contraceptives is challenging as well, often leading to small study samples. Hence, most of the studies investigate one particular menstrual cycle phase instead of doing research during both follicular and luteal phases. Although that is not necessarily a problem, it hinders our understanding of the potential impact of fluctuating hormones in women's health.

In *Chapter 4* we reported that women with BPD and CPD had distinct physiological responses to psychosocial stress, when tested during luteal phase. Although we have carefully defined the menstrual cycle phase during which we performed our studies, it is currently unclear whether the findings would be applicable during the follicular phase. In contrast to the luteal phase, the follicular phase is marked with more fluctuations. The early follicular phase is characterized by both low estradiol and progesterone levels, whereas the mid to late follicular phase is associated with markedly elevated estradiol concentrations (28). Ovarian hormone fluctuations

across the menstrual cycle have been shown to co-vary with stress resilience and changes in mood (29,30). Several studies have documented that when progesterone and estradiol levels decline after a relatively stable period of elevated concentrations, women experience more negative mood changes and are more vulnerable to stress (29,31,32). Furthermore, anger, sadness and irritability are the most commonly reported symptoms during the luteal phase in women with a premenstrual dysphoric disorder, suggesting that a proportion of women are more vulnerable to hormonal fluctuations (33). It might be hypothesized that some BPD features, such as extreme emotional instability and reactivity, are associated with cyclical hormone changes. Therefore, studies with clear operationally-defined periods of the menstrual cycle are needed in order to accurately differentiate between the effects of estradiol and progesterone, not only on psychophysiological stress reactivity, but also on the emotional, cognitive, and behavioral functioning of healthy women and women at high risk for psychopathology.

Hormonal contraception is another factor associated with negative effects on emotional, cognitive and behavioral properties. One of the most progressive developments in recent contraception policy has been the development of long-acting reversible contraception (34), such as implants or progesterone releasing intrauterine devices. Although the advantages of using an LNG-IUD are indisputable regarding its efficacy, safety, and local and rapid reversible nature, significant discontinuation rates have also been reported (35,36). *Chapter* 6 provides important experimental evidence that the LNG-IUD exerts robust systemic influences. We observed significantly elevated cortisol responses and a down-regulation of adrenal cortex function in healthy women using the LNG-IUD. Unfortunately, the design of the studies reported in *Chapter* 6 did not permit an assessment of whether the observed systemic physiological influences of LNG-IUD are associated with mood disturbances or emotional lability. Hence, taken together with the emerging evidence that progesterone influences the risk and severity of mood and anxiety disorders, and the rapidly increasing number of women using the LNG-IUD, the possibility that the LNG-IUD might impose a clinically-significant risk needs to be evaluated in large population-based observational studies. Although the side effects of hormonal contraceptives in general have been largely underreported, awareness of the adverse effects is important for both patients using hormonal contraceptives and for prescribing physicians. Satisfaction and continuation rates might be improved if health care professionals had access to more detailed research on side effects, and therefore could provide improved counseling.

Considering that the high prevalence of affective disorders, and increased stress sensitivity, in women is partly attributed to both endogenous and exogenous hormonal factors, we need to improve our understanding of these complex relationships. The challenge in understanding the significance of the vast array of stress reactivity

and hormone fluctuations for women's health and health care rests not so much in assessing the influence of each sex and stress hormone in isolation, but rather in understanding how these hormones interact throughout the course of the reproductive cycle. Improved knowledge of how stress hormones interact with sex hormones to contribute to stress resilience or vulnerability, and ultimately how such an interaction might contribute to etiology of stress-related disorders, might help offer new targets for therapies. Finally, women have the right to know the consequences of the hormonal changes that their body goes through during their lifetime, including sufficient facts to make an informed decision regarding choice of contraceptive method, and further research is vital to ensure this.

In conclusion, our investigation of different factors regarding cognitive processing and psychophysiological stress response provide evidence that:

- maladaptive personality traits are important factors in understanding the relationships between fundamental personality characteristics and cognitive processing during acute psychosocial stress, in both women with and without personality disorder;
- the 5-HTTLPR genotype is significantly associated with the cortisol response to acute psychosocial stress;
- the physiological response to acute psychosocial stress differs between groups with distinct personality psychopathology whereas the subjective mood disturbance response does not;
- distinct hormonal contraceptive methods have contrasting effects on physiological reactivity to acute psychosocial stress in healthy women;
- ACTH stimulation of the adrenal cortex elicits gonadotropin release in healthy premenopausal women.

While these explorations add important clues towards a more comprehensive understanding and coherent picture of stress induced sensitivity in women, it is apparent that sex hormones play an important role, and interact with a variety of factors, including fundamental personality traits, personality pathology, genetic factors, and environmental influences, to regulate physiological reactivity and adaptation to stress, and thereby women's individual well-being.

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Summary / Samenvatting

SUMMARY

Background and aims

Many of the stressors of our daily lives are psychological in nature and often socially oriented. Psychosocial stress is a reaction to a real or interpreted threat to the integrity of an individual that manifests itself by biochemical, physiological, cognitive and behavioral changes. It is commonly accepted that individuals vary markedly in the way they react to a challenging natural environment, or to complex social interactions. It is also widely acknowledged that these individual differences might have implications for behavior and health outcomes. According to the stress-diathesis theory, individual differences in reactivity to stressful events are dependent on personality characteristics that might either buffer, or be a predisposing risk factor, for emotional upheaval and ultimately risk of developing psychiatric decompensation. Considering that maladaptive emotional control is a significant burden in women with personality disorders, the aim of this thesis (described in chapter 1) was to investigate how stress regulation in women is modified by personality disorders through quantitative assessments of their psychophysiological response to acute psychosocial stress. We assessed cognitive appraisal and psychophysiological responses during a standardized psychosocial stress procedure, the Trier Social Stress Task (TSST). The TSST was conducted according to the original protocol reported by Kirshbaum et al. (1993) consisting of a preparation period, a free speech task and a verbal mental arithmetic task, each lasting 5 minutes. The TSST was performed in front of a two-member panel that maintained affectively neutral facial expressions throughout the procedure and provided the participant with no verbal or non-verbal feedback. We also examined genetic and hormonal factors that might contribute to the biology underlying physiological stress reactivity in women.

The studies were carried out at the department of Psychotherapy of the Riagg Rijnmond (Schiedam, The Netherlands) and the department of Psychiatry of the Erasmus University Medical Center (Rotterdam, The Netherlands). Study subjects were recruited among women with DSM-IV Axis II diagnosed personality psychopathology, who were under outpatient treatment at the department of Psychotherapy of the Riagg Rijnmond. Patients were considered ineligible to participate if they had a comorbid diagnosis of bipolar disorder, schizophrenia, current major depression, or had used psychotropic medication within the previous 9 months. The control group consisted of healthy adult women of reproductive age who were recruited from the community through local advertisements. Eligibility requirements for healthy controls included the absence of any DSM-IV Axis I or Axis II diagnoses, as well as no ongoing or previous psychiatric or psychological treatment. Questionnaires were completed

by all participants, yielding information on symptoms of psychopathology, childhood trauma, attachment, positive and negative affect, and general health.

All women participated in a psychosocial stress test during which heart rate and skin conductance level (SCL) were measured continuously, and salivary cortisol levels and subjective mood disturbance were measured at regular intervals. In addition, the serotonin transporter gene-linked polymorphic region (5-HTTLPR) and glucocorticoid receptor genotyping was performed in order to examine the association of candidate genetic factors on HPA axis reactivity to psychosocial stress. Cognitive appraisal was assessed during the anticipation period, directly before the performance of the stress task.

An additional study was performed in a cohort of healthy women with identical eligibility requirements as for the control group in the main TSST study. Low-dose (1µg) intravenous adrenocorticotropic hormone (ACTH) was administered in order to dissect the influence of stress on female reproductive physiology (gonadotropin release). We measured the concentration of hair cortisol to determine whether the laboratory-based assessments of HPA axis functioning during acute stress would also be confirmed by studying long-term cortisol exposure under naturalistic conditions.

Findings

In *chapter 2*, we examined the direct and indirect impact of attachment insecurity and temperament on the cognitive appraisals of acute psychosocial stress in a female sample consisting of healthy women and women with a personality disorder. In addition, the mediating role of maladaptive personality traits was taken into account. Our findings showed that positive affectivity was directly linked to secondary appraisal of acute psychosocial stress confirming the earlier suggestions that positive affectivity buffers against stress. Furthermore, we found that maladaptive personality traits mediated the negative impact of both attachment anxiety and negative affectivity on primary appraisal of acute psychosocial stress. Most importantly, this pattern of associations applied equally to women with personality disorder and healthy controls, confirming the importance of maladaptive personality traits for understanding the contribution of individual characteristics on cognitive appraisals of acute psychosocial stress.

Considering the growing evidence of a potential association between the serotonin transporter gene-linked polymorphic region (5-HTTLPR) and HPA axis functioning, *chapter 3* describes the outcome of our study designed to investigate how the HPA axis response to psychosocial stress is moderated in women by 5-HTTLPR genotype. In addition, we examined whether this association was moderated by the 5-HTTLPR interaction with experienced early life stress. We found that women carrying two copies of the long (LL) version of the 5-HTTLPR displayed exaggerated cortisol re-

sponses to psychosocial stress compared women with at least one copy of the short (SL or SS) allele. This association did not change when a potential interaction of 5-HTTLPR genotype and early life adversity was taken into account. Our findings demonstrate the complex association between the 5-HTTLPR and cortisol reactivity to psychosocial stress, for which additional studies will be required to further clarify the relationships between genetic predisposition and stress sensitivity.

In *chapter 4*, we investigated whether the psychophysiological stress response differs as a function of personality disorder diagnosis. We compared subjective mood disturbance, heart rate, SCL, and salivary cortisol responses to psychosocial stress in women with cluster C personality disorder (CPD) and borderline personality disorder (BPD). Both CPD and BPD patients reported a similar burden of subjective mood disturbance after performing the TSST. However, only BPD patients demonstrated reduced baseline cortisol levels with a blunted cortisol and heart rate response to the TSST. In addition, BPD patients exhibited a generalized increase of SCL. No significant differences in baseline or TSST reactivity of cortisol, heart rate, or SCL were observed between CPD patients and healthy controls. Therefore, we concluded that BPD patients have a distinct psychophysiological responsivity to psychosocial stress, indicating a potentially distinct underlying biology.

Although the use of hormonal contraception among women is increasing annually, our knowledge about the effects of contraception on stress-induced physiology in women is, remarkably, very limited. In particular, data on long-acting contraceptives such as the progestin releasing intrauterine device (LNG-IUD) is almost entirely unexplored. Therefore, in *chapter 5*, we investigated the effects of hormonal contraception on female stress physiology. We found that women using the LNG-IUD had an exaggerated salivary cortisol response to the TSST, compared to women using combined oral contraceptives and natural cycling women. Heart rate responses were also significantly potentiated during the TSST in women using a LNG-IUD. After ACTH challenge, women using the LNG-IUD or combined oral contraceptives had a blunted salivary cortisol response compared to naturally cycling women. In line with the TSST findings, women using the LNG-IUD had significantly elevated levels of hair cortisol. Although the LNG-IUD has been widely reported to function with negligible systemic effects, our findings suggest that LNG-IUD contraception induces a centrally-mediated sensitization of both autonomic and hypothalamic-pituitary-adrenal (HPA) axis responsivity. We concluded that large population-based observational studies are urgently warranted to evaluate the potential risk of the LNG-IUD for developing mood and anxiety disorders.

Previous studies in postmenopausal women have demonstrated that the gonadotropin response to adrenal stimulation is highly estrogen-dependent, and significantly potentiated by progesterone. In *chapter 6,* we investigated the effects of acute stress

on gonadotropin release in premenopausal women. We have examined this effect in 3 independent groups defined by their differential use of hormonal contraceptives: 1) women having a natural menstrual cycle, 2) women using oral contraceptives (combination estrogen/progestin), and 3) women using an LNG-IUD. With this study design, we were able to further dissect the hormonal context by which adrenal cortex activity mediates gonadotropin release. Our results confirmed a permissive function of estradiol and demonstrated that acute stimulation of adrenal steroids, most likely cortisol, mediates gonadotropin release.

In *chapter 7*, the main results and conclusions of this thesis were presented and discussed. We have gained further insight into the psychophysiological responses to stress, and defined important determinants that influence these responses in women with and without personality disorder. We showed that when exposed to a challenging situation, cognitive perceptions of stress are strongly and directly influenced by emotional dysregulation, and indirectly by varying influences of attachment style and temperament. Furthermore, we provided evidence that maladaptive personality traits are important factors in understanding the relationships between attachment, temperament and mentalization capacity during acute psychosocial stress, not only in clinical samples, but also in the general population. Another important finding was the role of genetic factors in the physiological response to stress in women of reproductive age. We found that women with the LL genotype of the 5-HTTLPR polymorphism demonstrated significantly higher salivary cortisol responses to psychosocial stress compared to women with at least one copy of the S allele. Furthermore, our data indicated that the physiological stress response differs as a function of the subtype of personality disorder. Although women suffering from either BPD or CPD exhibited similar levels of mood disturbance in response to psychosocial stress, patients with BPD demonstrated significant attenuations of cortisol and heart rate reactivity compared to patients with CPD or healthy controls. Moreover, our findings indicated that the pattern of blunted cortisol and heart rate reactivity to psychosocial stress was specific to patients with BPD, rather than simply a consequence of emotional vulnerability in personality psychopathology. Regarding the impact of sex hormones, we found that distinct hormonal contraceptive methods have contrasting effects on endocrine reactivity to acute psychosocial stress. Compared to natural cycling women, women using an LNG-IUD exhibited a robust potentiation of the salivary cortisol response during the TSST, whereas women using combination estrogen-progestin contraception showed a blunted cortisol response. Similarly, women using the LNG-IUD showed significantly higher concentrations of hair cortisol than naturally cycling women. Lastly, our data demonstrates that acute stimulation of adrenal steroid production, most likely cortisol, mediates enhanced gonadotropin release in healthy premenopausal women.

Taken all together, these explorations of varying determinants of psychophysiological responses to psychosocial stress provide important clues in establishing a more comprehensive understanding of stress induced sensitivity in women of reproductive age with and without personality psychopathology. Considering that stress sensitivity is frequently investigated as a vulnerability marker for both mental and physical health problems, we argue that circulating sex hormone levels should be taken into consideration while examining the responses to psychosocial stress in women.

SAMENVATTING

Achtergrond en doelstellingen

Veel van de stressoren die we in ons dagelijks leven ervaren zijn psychologisch van aard en vaak sociaal van oorsprong. Psychosociale stress kan worden gedefinieerd als een reële of een ervaren bedreiging van de psychische integriteit van een individu die gepaard gaat met biochemische, fysiologische, cognitieve en gedragsmatige veranderingen. Het is algemeen geaccepteerd dat individuen aanzienlijk verschillen in de wijze waarop zij reageren op hun omgeving of op complexe sociale interacties. Ook is het algemeen aanvaard dat deze individuele verschillen gevolgen kunnen hebben voor gedrag en gezondheid. Volgens het stress-kwetsbaarheidsmodel zijn individuele verschillen in reactiviteit op stressvolle gebeurtenissen afhankelijk van persoonlijkheidskenmerken. Persoonlijkheidskenmerken kunnen bij stress een beschermende factor zijn voor emotionele ontregeling of, in geval van kwetsbaarheid, emotionele ontregeling juist faciliteren. Uit het model volgt dat hoog kwestbare individuen onder invloed van stressoren psychiatrische ziekten kunnen ontwikkelen.

Het doel van dit proefschrift is de stressgevoeligheid te onderzoeken bij vrouwen met en zonder persoonlijkheidsstoornis door hun psychofysiologische reacties op acute psychosociale stress te bestuderen. We onderzochten (hoofdstuk 1) de 'cognitive appraisals' (subjectieve evaluatie van ernst van de stressor en de eigen weerbaarheid ertegen) cognitieve "appraisals" en de psychofysiologische responsiviteit tijdens een psychosociale stressprocedure, de Trier Sociale Stress Taak (TSST). De TSST is uitgevoerd volgens het oorspronkelijke protocol zoals opgesteld door Kirshbaum et al. (1993). Het stressprotocol bestaat uit een voorbereidingsperiode, een opdracht voor een fictief sollicitatiegesprek en een verbale hoofdrekentaak van elk 5 minuten. De TSST werd uitgevoerd ten overstaan van een tweeledig panel dat gedurende de hele procedure geen enkele gezichtsuitdrukking toonde of (non)verbale feedback gaf. Daarnaast onderzochten we de genetische en hormonale factoren die een rol spelen in de fysiologische stressreactiviteit van vrouwen.

De studies werden uitgevoerd op de afdeling Psychotherapie van de Riagg Rijnmond (Schiedam, Nederland) en de afdeling Psychiatrie van Erasmus Universitair Medisch Centrum (Rotterdam, Nederland). In deze studies hebben wij twee steekproeven van vrouwelijke proefpersonen onderzocht. De ene steekproef bestond uit ambulante patiënten met persoonlijkheidspsychopathologie, die op de afdeling Psychotherapie van Riagg Rijnmond in behandeling waren. Patiënten met een comorbide diagnose van een bipolaire stoornis, schizofrenie of depressie, of die in de afgelopen 9 maanden psychotrope medicijnen hadden gebruikt, waren uitgesloten van deelname. De andere steekproef bestond uit gezonde volwassen vrouwen van vruchtbare leeftijd (18-46 jaar). Voor deze laatste steekproef werd geworven onder de algemene bevol-

king door het plaatsen van lokale advertenties. Criteria voor deelname van gezonde proefpersonen waren: geen DSM-IV as-I of as-II diagnose hebben en niet onder psychiatrische of psychologische behandeling zijn of ooit geweest zijn.

Gedurende de psychosociale stressprocedure werd van alle vrouwen de hartslag en het huidgeleidingsniveau continu gemeten en werden met regelmatige tussenpozen cortisolniveaus in speeksel bepaald en werd de subjectieve veranderingen in de gemoedstoestand gemeten door gebruik van een korte vragenlijst. Het serotonine transporter gen promoter polymorfisme (5-HTTLPR) werd bepaald om de rol van deze genetische factor op de reactiviteit van de hypothalamus-hypofyse-bijnier (HPA) as op psychosociale stress te onderzoeken. De cognitieve appraisal werd beoordeeld tijdens de voorbereidingstijd, direct vóór de uitvoering van de stress taak. Bij alle proefpersonen werd een selectie van vragenlijsten afgenomen, gericht op het meten van symptomen van psychopathologie, jeugdtrauma, gehechtheidsstijl, positieve en negatieve affectiviteit en algemene gezondheid. Om de effecten van stress op de vrouwelijke fysiologie (gonadotropine vrijlating) te onderzoeken werd een aanvullende studie uitgevoerd waarbij gezonde vrouwen met verschillende anticonceptie methode een lage dosis (1µg) adrenocorticotropic hormoon (ACTH) intraveneus toegediend kregen. Tot slot hebben we de cortisolconcentratie in het haar van de gezonde vrouwen bepaald. Dit om te onderzoeken of de laboratorium bevindingen betreffende de effecten van anticonceptie op de HPA as reactiviteit overeenkomen met de cortisolconcentraties gedurende een langere periode tijdens normale ambulante omstandigheden.

Bevindingen

We hebben de directe en de indirecte gevolgen van een onveilige gehechtheidsstijl en van temperament op de cognitieve appraisal van acute psychosociale stress onderzocht in een steekproef van gezonde vrouwen en vrouwen met een persoonlijkheidsstoornis (hoofdstuk 2). Daarbij werd rekening gehouden met de modulerende rol van niet-adaptieve persoonlijkheidstrekken. Uit onze bevindingen bleek dat positieve affectiviteit direct gekoppeld is aan secundaire appraisals van acute psychosociale stress. Deze bevinding bevestigt de al eerder gesuggereerde beschermende rol van positieve affectiviteit tegen stressoren. Bovendien vonden we dat niet-adaptieve persoonlijkheidstrekken de effecten van zowel de aan gehechtheid gerelateerde angst als de negatieve affectiviteit op de primaire appraisal van acute psychosociale stress medieren. Dit patroon van associaties gaat op voor zowel de gezonde vrouwen als voor vrouwen met persoonlijkheidspsychopathologie. De bevindingen suggereren dat niet adaptieve persoonlijkheidstrekken belangrijke factoren zijn om het effect van individuele kenmerken op cognitieve appraisals van acute psychosociale stress beter te begrijpen.

In hoofdstuk 3 hebben we onderzocht of de reactiviteit van de HPA as op psychosociale stress wordt gemoduleerd door de 5-HTTLPR in een groep van vrouwelijke proefpersonen. Bovendien onderzochten we of deze associatie beïnvloed wordt door de interactie van de 5-HTTLPR en het hebben meegemaakt van een jeugdtrauma. In tegenstelling tot de bekende literatuur, vonden we dat vrouwen die twee exemplaren van de lange (LL)-allelen van de 5-HTTLPR dragen een hogere cortisolrespons op psychosociale stress lieten zien dan vrouwen met ten minste één kopie van de korte (SL of SS)-allelen. Verder veranderde deze waargenomen associatie niet significant wanneer de interactie tussen 5-HTTLPR and jeugdtrauma werd meegerekend. Onze bevindingen laten de complexe associatie zien tussen de 5-HTTLPR en de cortisol reactiviteit op psychosociale stress. Er zijn meer studies onder vrouwen nodig om de relatie tussen genetische aanleg en stress gevoeligheid verder te verduidelijken.

Ook is onderzocht of de psychofysiologische stressrespons op psychosociale stress verschilt per soort persoonlijkheidspsychopathologie (hoofdstuk 4). We vergeleken de subjectieve gemoedstoestandverandering, hartslag, huidgeleiding en cortisolresponsen op psychosociale stress bij vrouwen met een cluster C persoonlijkheidsstoornis (CPD) of een borderline persoonlijkheidsstoornis (BPD). Zowel CPD als BPD patiënten lieten meteen na het uitvoeren van de stresstaak een vergelijkbare toename in subjectieve gemoedstoestandverandering zien. Echter, patiënten met BPD lieten zowel een lagere baseline van cortisolniveaus zien als een lagere cortisol en hartslag reactiviteit op de TSST. Daarnaast lieten patiënten met BPD verhoogde niveaus van huidgeleiding zien. Er zijn geen significante verschillen in baseline of reactiviteit van cortisol, hartslag of huidgeleiding op de TSST waargenomen tussen CPD patiënten en gezonde vrouwen. We vonden dus dat BPD patiënten, in tegenstelling tot de CPD patiënten, een andere psychofysiologische responsiviteit op psychosociale stress hebben, wat een verschillende onderliggende biologie van de psychopathologie suggereert.

Hoewel het gebruik van hormonale anticonceptie onder vrouwen jaarlijks groeit, is onze kennis over de effecten van anticonceptie op stress-geïnduceerde fysiologie bij vrouwen zeer beperkt. Met name de effecten van langdurig gebruik van voorbehoedsmiddelen (zoals een hormonaal spiraal, geplaatst in baarmoeder; LNG-IUD) zijn onbekend. Wij onderzochten daarom de impact van hormonale anticonceptie op de vrouwelijke stressfysiologie (hoofdstuk 5). We vonden dat vrouwen die een LNG-IUD gebruikten een significant hogere cortisolrespons lieten zien in reactie op de TSST, vergeleken met vrouwen die een gecombineerde orale anticonceptie (pil) gebruikten en vrouwen met een natuurlijke menstruele cyclus. Ook de hartslag was aanzienlijk verhoogd tijdens de TSST bij vrouwen die een LNG-IUD gebruikten. Na de ACTH test lieten vrouwen met een LNG-IUD en vrouwen die de pil gebruikten een verlaagde cortisolrespons zien vergeleken met vrouwen met een natuurlijke menstruele cyclus.

In overeenstemming met de TSST bevindingen lieten vrouwen met een LNG-IUD aanzienlijk verhoogde niveaus van haarcortisol zien vergeleken met vrouwen met een anticonceptie pil of een natuurlijke menstruele cyclus. Onze bevindingen zijn een sterke aanwijzing dat een LNG-IUD een systemisch werkingsmechanisme heeft, terwijl de bijsluiters van dit type anticonceptie een lokale werking suggereren met verwaarloosbare systemische bijwerkingen.

Studies onder postmenopauzale vrouwen concludeerden dat de gonadotropine reactie op bijnierstimulatie zeer estradiolafhankelijk is en significant gestimuleerd wordt door progesteron niveaus. Wij onderzochten de effecten van acute stress op de afgifte van gonadotropinen in een steekproef van premenopausale vrouwen (hoofdstuk 6). We hebben dit effect onderzocht in drie onafhankelijke groepen die waren gedefinieerd op basis van gebruik van hormonale anticonceptie: 1) vrouwen met een natuurlijke menstruatiecyclus, 2) vrouwen die orale anticonceptie gebruikten (combinatie oestrogeen/progestageen) en 3) vrouwen die een LNG-IUD gebruikten. Met dit studiedesign konden we de effecten van de hormonale context en de stimulatie van bijnierschors op de gonadotropinenrespons verder ontrafelen. Onze bevindingen bevestigen een belangrijke rol van estradiol en tonen aan dat acute stimulatie van bijniersteroïden, hoogstwaarschijnlijk cortisol, de afgifte van gonadotropinen beïnvloeden.

Discussie en conclusies

Het proefschrift geeft meer inzicht in de psychofysiologische reacties op stress en de meest bepalende factoren die van invloed zijn op deze psychofysiologische reacties bij vrouwen met en zonder persoonlijkheidspsychopathologie (hoofdstuk 7). We toonden aan dat wanneer vrouwen worden blootgesteld aan een stressvolle situatie, de betekenis van de cognitieve perceptie van stress wordt versterkt door de invloed van emotionele disregulatie, en indirect door de invloeden van gehechtheidsstijl en temperament. Bovendien bewijzen we dat niet-adaptieve persoonlijkheidstrekken belangrijke factoren zijn om inzicht te krijgen in de relaties tussen gehechtheidsstijl, temperament en mentalizerend vermogen tijdens acute psychosociale stress, niet alleen binnen de klinische populatie, maar ook onder de algemene bevolking. Een andere belangrijke vaststelling is de rol van genetische factoren in de stressfysiologie bij vrouwen. We hebben aangetoond dat vrouwen met het genotype LL van de 5-HTTLPR-polymorfisme aanzienlijk hogere cortisolreacties op psychosociale stress hebben dan vrouwen met ten minste één kopie van het S-allel. Bovendien laten we zien dat de fysiologische stressrespons verschilt per persoonlijkheidspsychopathologie. Hoewel vrouwen die lijden aan BPD of CPD een overeenkomstige gemoedstoestandverandering vertonen als gevolg van psychosociale stress, laten patiënten met BPD een aanzienlijk lagere cortisol- en hartslagreactiviteit zien ten opzichte

van patiënten met CPD of gezonde vrouwen. Bovendien tonen deze bevindingen aan dat dit patroon van verlaagde cortisol- en hartslagreactiviteit op psychosociale stress specifiek is voor patiënten met BPD in plaats van eenvoudigweg een gevolg van emotionele kwetsbaarheid bij de persoonlijkheidpsychopathologie.

Met betrekking tot de invloed van geslachtshormonen vonden we dat verschillende hormonale anticonceptie verschillende effecten hebben op de fysiologische responsiviteit op acute psychosociale stress. Vergeleken met vrouwen met natuurlijke menstruele cyclus, lieten vrouwen die een LNG-IUD gebruikten een grote toename van de cortisolrespons zien in reactie op de TSST, terwijl vrouwen die een combinatie anticonceptie gebruikten een verlaagde cortisolrespons lieten zien. Ook bleek dat vrouwen die een LNG-IUD gebruikten aanzienlijk hogere concentraties van haarcortisol hadden dan vrouwen met een natuurlijke menstruele cyclus. Tot slot laten onze gegevens zien dat acute stimulatie van bijniersteroïden, hoogstwaarschijnlijk cortisol, de afgifte van gonadotropinen beïnvloeden bij gezonde premenopauzale vrouwen.

Samengevat zijn deze studies belangrijke stappen in het verkrijgen van inzicht in een meer omvattend en samenhangend beeld van stressgevoeligheid bij vrouwen van vruchtbare leeftijd met en zonder persoonlijkheidspsychopathologie. Omdat stressgevoeligheid vaak is onderzocht als een kwetsbaarheidsfactor voor zowel mentale als fysieke gezondheidsproblemen, stellen we dat er bij vrouwen rekening moet worden gehouden met geslachtshormoonniveaus bij onderzoek naar de psychofysiologische reacties op psychosociale stress.

PHD PORTFOLIO

Name PhD student: J. Aleknaviciute
Erasmus MC Department: Psychiatry
PhD period: September 2010 – September 2016

Promotor: Prof. dr. S.A. Kushner
Co-promotor 1: Dr. J.H.M. Tulen
Co-promotor 2: Dr. C.G. Kooiman

PhD training	Year	Workload (ECTS)*
General academic skills		
Research integrity (Erasmus MC)	2011	2
Basis cursus regelgeving en organisatie voor klinische onderzoekers (BROK), /hercertificering	2011/2016	1
Writing a Scientific Article (Taalcentrum-VU)	2014	3
CPO minicursus: Methodologie voor Patiëntgebonden Onderzoek en Voorbereiding van Subsidieaanvragen	2012	0,3
Research Skills and courses		
Introduction to Data-analysis (NIHES)	2011	0,7
Principles of Research in Medicine (NIHES)	2011	0,7
Regression analysis (NIHES)	2012	1,4
Reproductive endocrinology, European Society of Endocrinology (ESE), Edinburgh	2015	2
Didactic skills		
Basistraining SCID I en SCID II (de Viersprong)	2011	1
Basis training didaktiek Teach the Teacher I (Desiderius School)	2014	0,6
Workshop 'omgaan met groepen' (desiderius School)	2015	0,2
Conferences and symposia		
International Conference on Attachment, Neuroscience and Emotions, Kaatsheuvel.	2011	0,7
5th International Conference on The (non)expression of Emotions in Health and Disease, Tilburg.	2011	0,7
52nd annual meeting of Society for Psychophysiological Research (SPR), Lousiana, USA. (poster presentation)	2012	1
43rd Annual meeting of ISPNE (International Society of Psychoneuroendocrinology). (poster presentation)	2013	1
53rd SPR annual meeting, Florence. (poster presentation)	2013	1
The European Society of Contraception and Reproductive Health (ESC). First Global Conference on Contraception, Reproductive and Sexual Health, Copenhagen.	2013	0,7
Methorstsymposium, "Onderzoek bij de Riagg. Hoe bestaat het?!", Vlaardingen. (oral presentation)	2013	0,2
Voorjaarscongres, Maastricht. (oral presentation)	2014	0,3

44th Annual meeting of ISPNE, Montreal, Canada. (poster presentation)	2014	1
Symposium, Nederlandse Vereniging voor Neuropsychoanalyse (NVNPSA), Amersfoort. (oral presentation)	2014	0,3
Symposium, "Contraception, meeting women's contraceptive needs", Gent.	2015	0,3
International society for the study of personality disorders (ISSPD), Montreal, Canada. (oral presentation)	2015	1
Teaching activities		
Tutor 1st year medical students	2011	2
2de jaars keuzeonderwijs	2012-2016	2,5
Workshop Master of Neuroscience	2012-2016	0,4
Supervising Master's theses (7 MSc theses, 3 BSc theses)	2011-2016	17

*1 ECTS (European Credit Transfer System) equals a workload of 28 hours

CURRICULUM VITAE

Jurate Aleknaviciute was born in Šilutė, Lithuania, on October 18, 1979. After finishing her secondary education at the Šilutės pirmoji gimnazija, in Šilutė, she started to study Economics at the University of Applied Sciences in Vilnius. In 2001, she obtained her Bachelor degree in Economics. In the fall of 2003 she moved to live in the Netherlands. She enrolled to study Psychology at the Erasmus University Rotterdam in 2004, where she obtained her Bachelor degree in Biological and Cognitive Psychology. Subsequently, she performed her Master program in Biological Psychology at the faculty of Psychology and Neuroscience of the University of Maastricht. During the specialization phase of her Master project, she focused on stress induced physiology, by studying mismatch negativity during psychosocial stress in a student sample; a study performed in collaboration with a fellow student at the Erasmus University in Rotterdam. In 2009, she started her psychology internship at the department of Psychiatry of the Erasmus MC where she obtained her BAPD (Basis Aantekening Psychodiagnostiek) in 2010. In that same year she started her PhD research on stress sensitivity in patients with and without a personality pathology at the department of Psychiatry of the Erasmus MC in Rotterdam, in collaboration with the department of Psychotherapy of the Riagg Rijnmond in Schiedam. The results of this project are described in this thesis. This research was carried out under the supervision of Dr. J.H.M. Tulen, Dr. C.G. Kooiman and Prof. Dr. S.A. Kushner.

PUBLICATIONS

Aleknaviciute J, Tulen JHM, Kamperman AM, de Rijke YB, Kooiman CG, Kushner SA. Borderline and cluster C personality disorders manifest distinct physiological responses to psychosocial stress. Psychoneuroendocrinology. 2016;*72*:131-138.

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DANKWOORD

Ik zie mijn promotietraject als een lange reis. Voor mijn vertrek las ik verschillende verslagen en luisterde ik naar reisverhalen. Zo kreeg ik een idee van wat ik te zien zou krijgen, proeven en ervaren. Ik werd gewaarschuwd voor bergen werk, geattendeerd op de gevaarlijke hoeken en gaten, en ook gewezen op zeeën van mogelijkheden. Uiteraard werd ik ook geïnformeerd over de moeilijkheidsgraad van de trail, gevolgd door een grote prijs, wat alle inspanning doet vervagen. Ik zie mijn proefschrift als het reisverslag van het onderzoekstraject dat ik heb afgelegd.

Er zijn 3 belangrijke ingrediënten die mijn avontuur, de voldoening en het succes van mijn reis hebben bepaald: 1) 'het Consulaat', een multifunctioneel opererend corps dat de reis mogelijk maakte, stuurde, van commentaar en advies voorzag en dat kennis en inzichten deelden; 2) de reisgenoten, mijn collega's, vrienden, patiënten en de proefpersonen, en anderen die (on)verwacht mijn pad kruisten. Zij maakten met hun ervaringen, inzichten, wel of niet in samenwerking mijn reis rijker en turbulenter; en 3) de 'backpack', mijn basis met mijn normen en waardesysteem, kennis en ervaring.

Ik heb de afgelopen jaren veel gereisd naar allerlei uithoeken van de wereld. Het reizen heeft mij ontwikkeld als persoon en mijn blik verruimd. Ook de wetenschappelijke reis die ik heb afgelegd heeft mij veel gebracht. Mijn 'backpack' werd tijdens deze reis een stuk compacter, functioneler, efficiënter en vooral comfortabeler. Met deze 'backpack' durf ik nu andere/nieuwe avontuurlijke reizen aan. Juist hiervoor ben ik heel dankbaar aan mijn Consulaat: mijn promotor professor Dr. Steven Kushner, en mijn copromotoren Dr. Joke Tulen en Dr. Kees Kooiman.

Beste Joke, Steven en Kees, mijn dank aan jullie is heel groot voor het vertrouwen die jullie in mij hebben gesteld. Ik ben dankbaar dat ik op deze ontdekkingsreis heb mogen gaan en vooral voor de mogelijkheid om van het uitgestippelde pad te mogen afwijken. Ik waardeer dat jullie mij de mogelijkheid hebben gegeven om onbekende gebieden te kunnen verkennen. Jullie hebben mij alle nodige bouwstenen aangereikt, niet alleen voor een volmaakte reis maar ook voor het schrijven van mijn reisverslag, mijn proefschrift. Ik kijk voldaan en trots terug op een verrijkende en leerzame reis.

Een land waar ik nog nooit ben geweest, geeft mij een heerlijke roes en nodigt mij uit om te proeven en te ervaren. In een onbekend land ben ik dankbaar als ik begeleid wordt door een 'local'. Mijn speciale dank gaat naar professoren Dr. Elisabeth van Rossum en Dr. Frank de Jong, voor het helpen navigeren en exploreren in het voor mij vrij onbekende endocrinologie land.

Voor de toegang tot een ander onbereisde regio, het gebied van epidemiologie, spreek ik mijn dankbaarheid uit aan professor Dr. Henning Tiemeier. Epidemiologie

was voor mij een bijzondere bestemming, niet zozeer als een plek, maar als een nieuwe manier om dingen te zien. De vruchten zijn aan het rijpen.

Ik voel me vereerd dat jullie een plaats willen innemen in mijn lees- en promotiecommissie, en in het bijzonder dat u, Frank, als een rector magnificus de plechtigheid wilt voorzitten. Dit vind ik heel speciaal. Ook mijn dank aan de andere commissie leden voor hun belangstelling, tijd, input en hun aanwezigheid bij mijn promotie.

Deze reis heb ik niet alleen ondernomen. Bedankt aan al mijn reisgenoten en maatjes die, bewust of onbewust, bereid waren een korter of langer stuk weg met mij samen af te leggen. Door jullie heeft mijn reis meer kleur en energie gekregen.

Zoals Michael Palin zei: "Als het reisvirus toeslaat, bestaat daar geen enkel medicijn voor. En ik weet dat ik tot mijn dood een blije patiënt zal zijn." Ik geloof dat een soortgelijk wetenschapsvirus ook bestaat en ik vrees dat ik daar zelf mee besmet ben.